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- (54) **VAGINAL INSERTED ESTRADIOL
PHARMACEUTICAL COMPOSITIONS AND
METHODS**
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(57) **ABSTRACT**

In one aspect, pharmaceutical compositions and methods for
the treatment of vulvovaginal atrophy (VVA) are provided.
In one embodiment, the method comprises digitally insert-
ing into the lower third of the vagina of a subject having
VVA a soft gelatin capsule containing a liquid pharmaceu-
tical composition.

7 Claims, 14 Drawing Sheets

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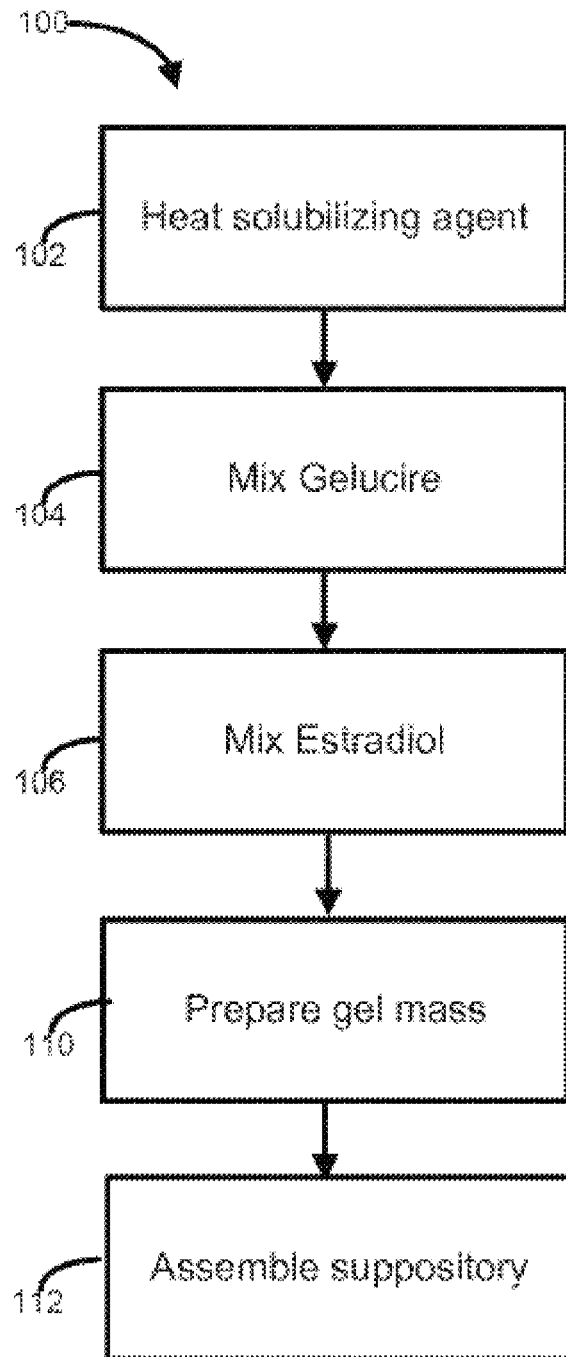


FIG. 1

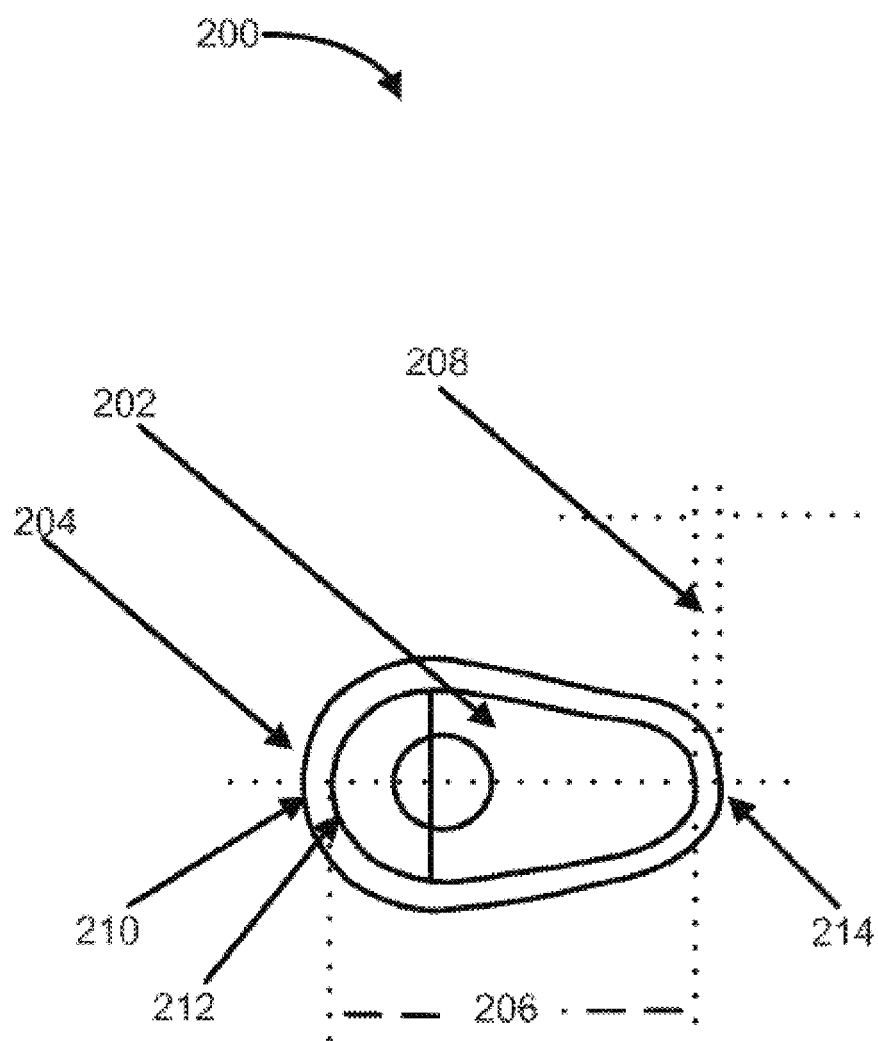


FIG. 2

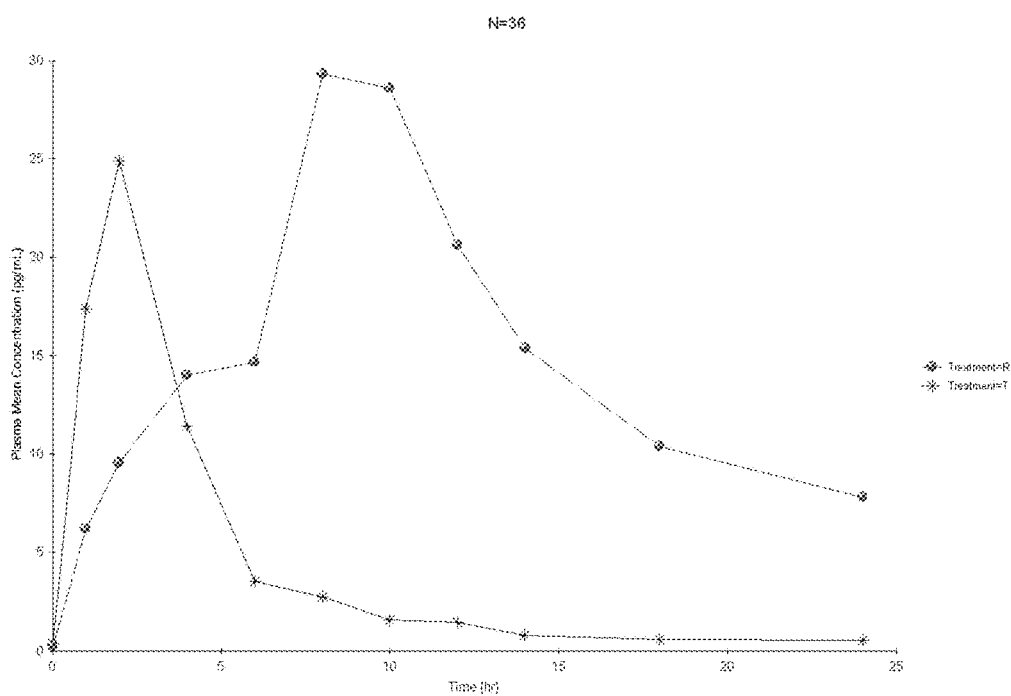


FIG. 3

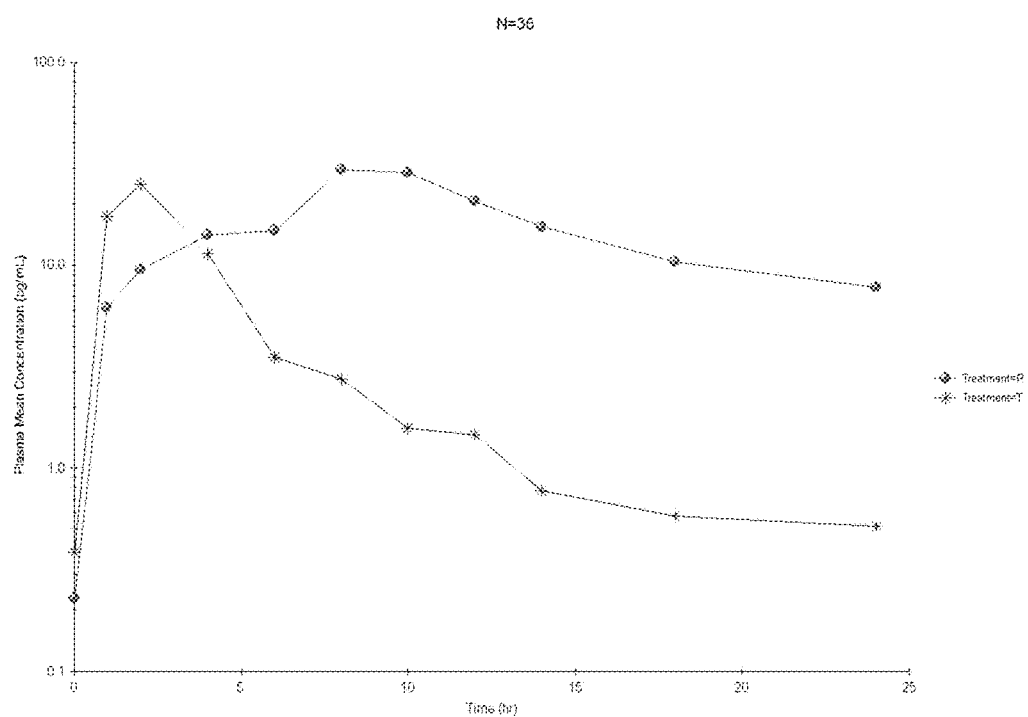


FIG. 4

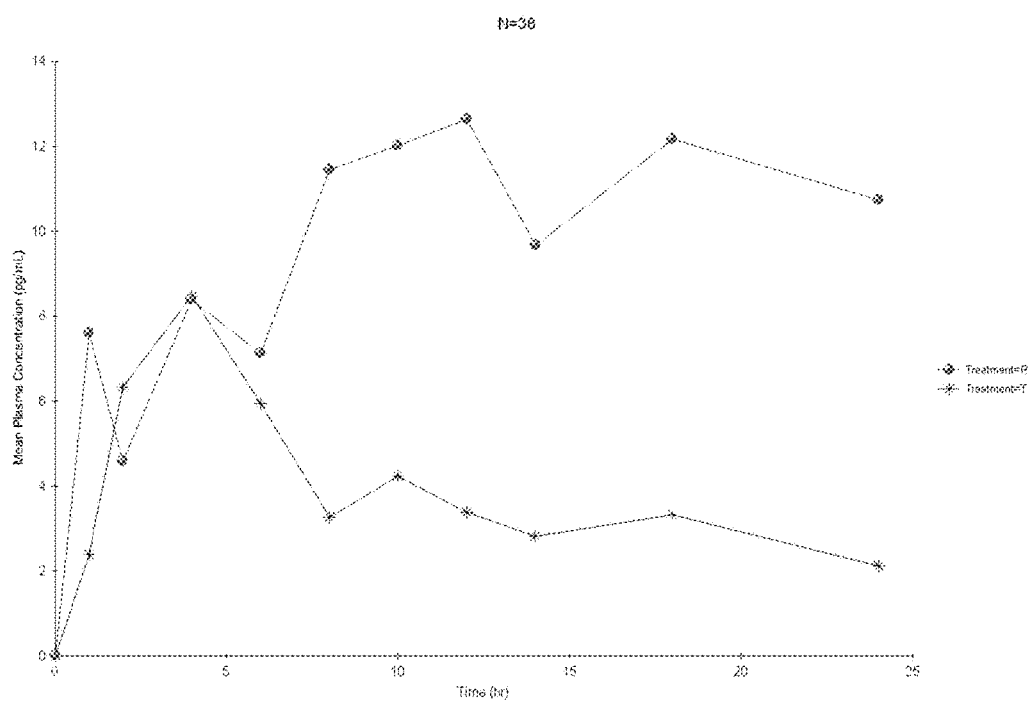


FIG. 5

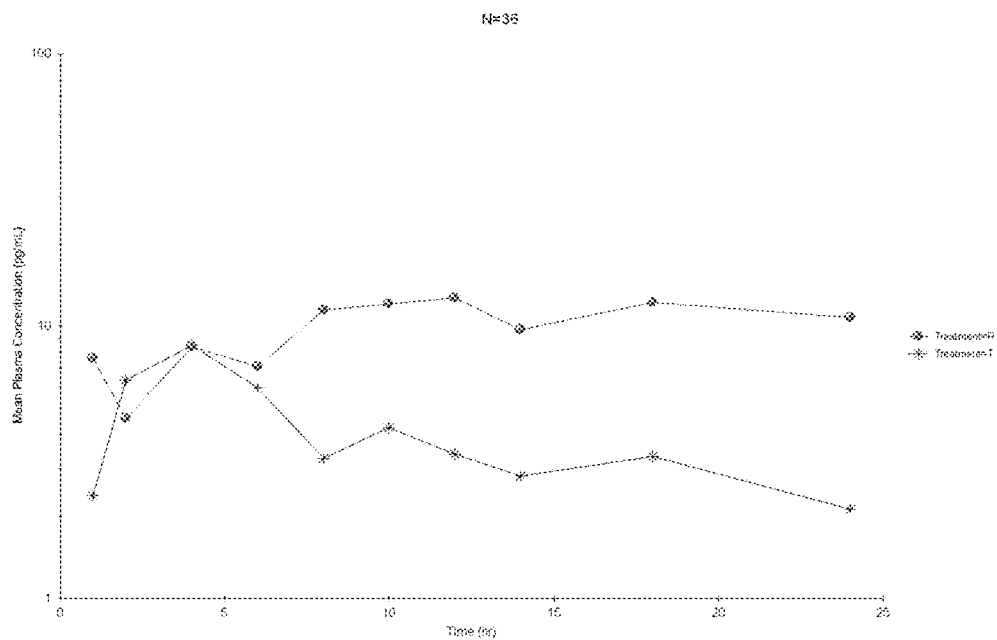


FIG. 6

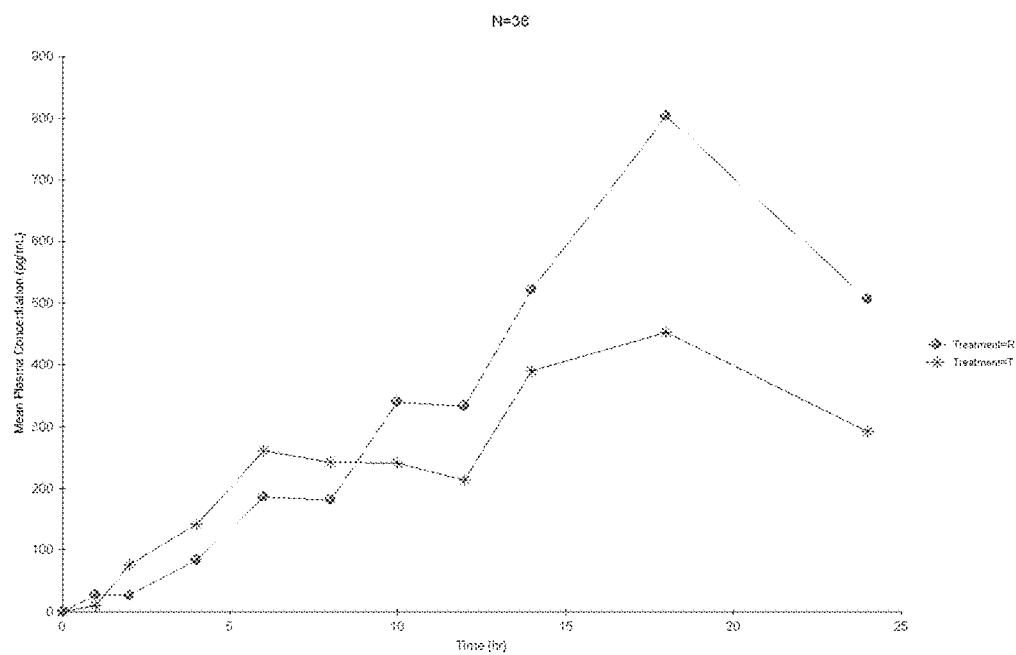


FIG. 7

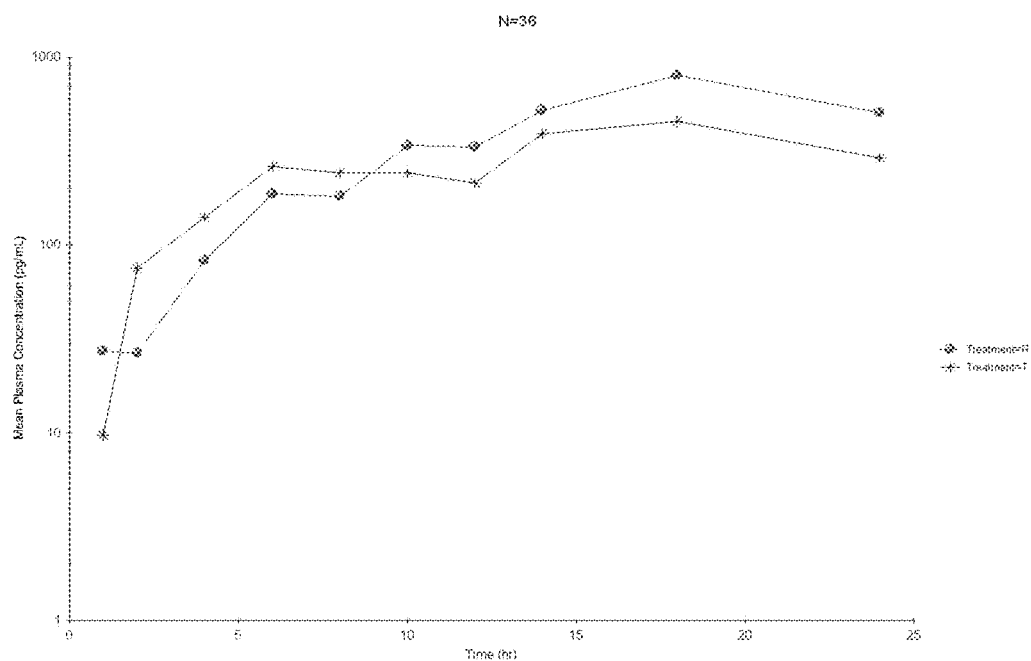


FIG. 8

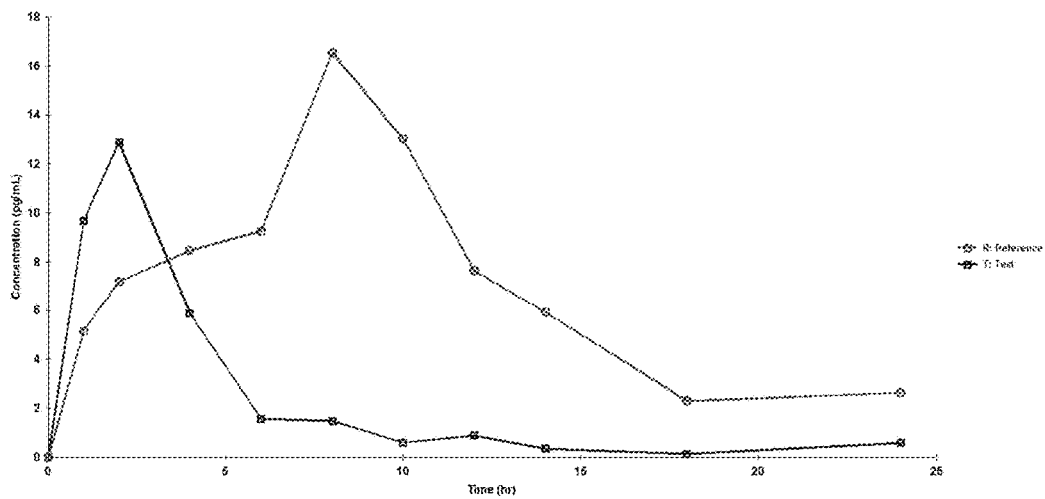


FIG. 9

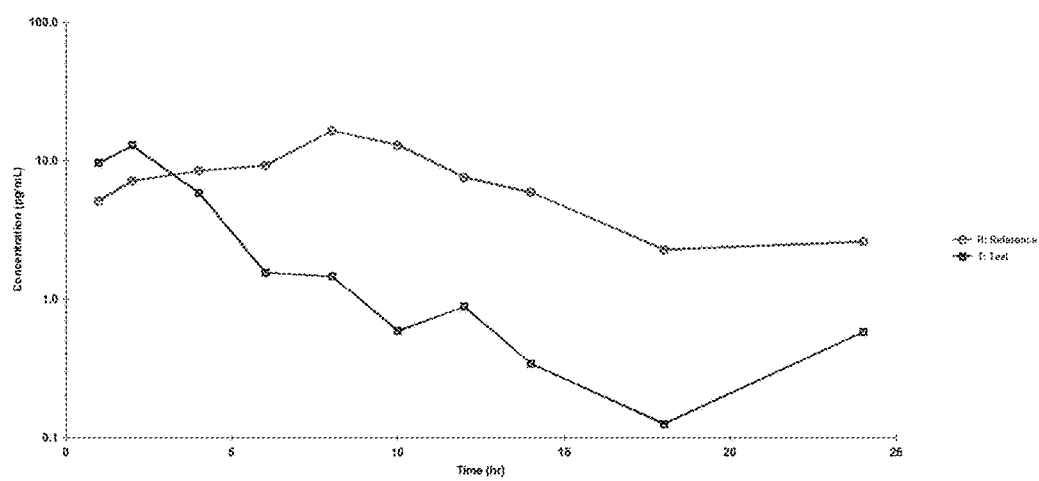


FIG. 10

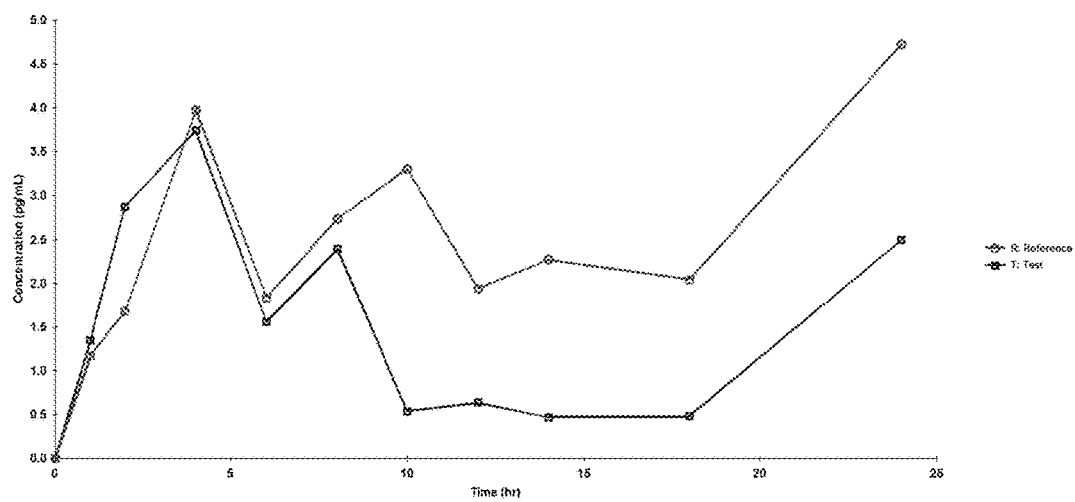


FIG. 11

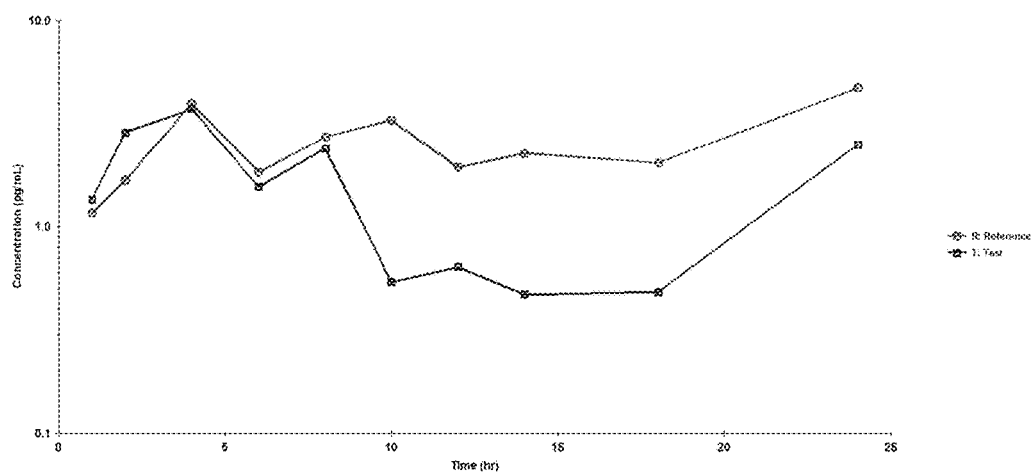


FIG. 12

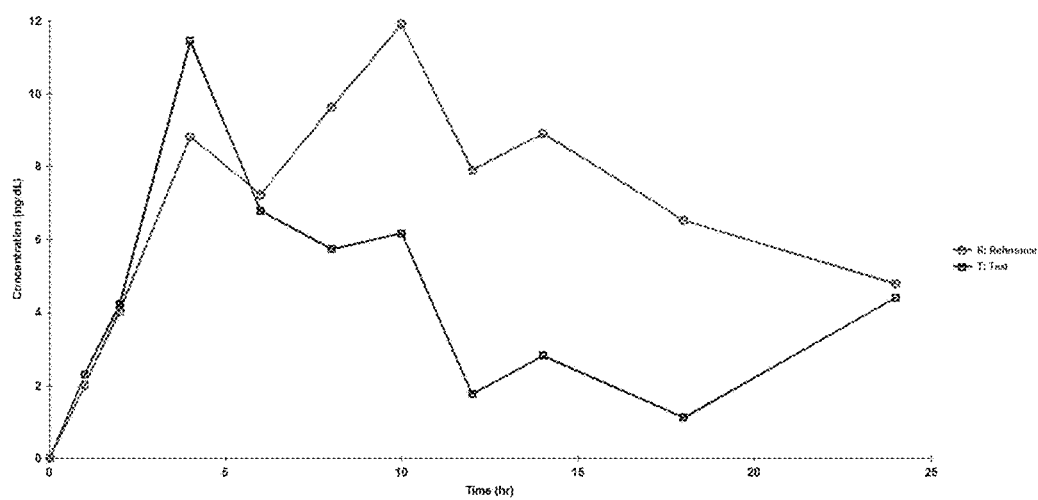


FIG. 13

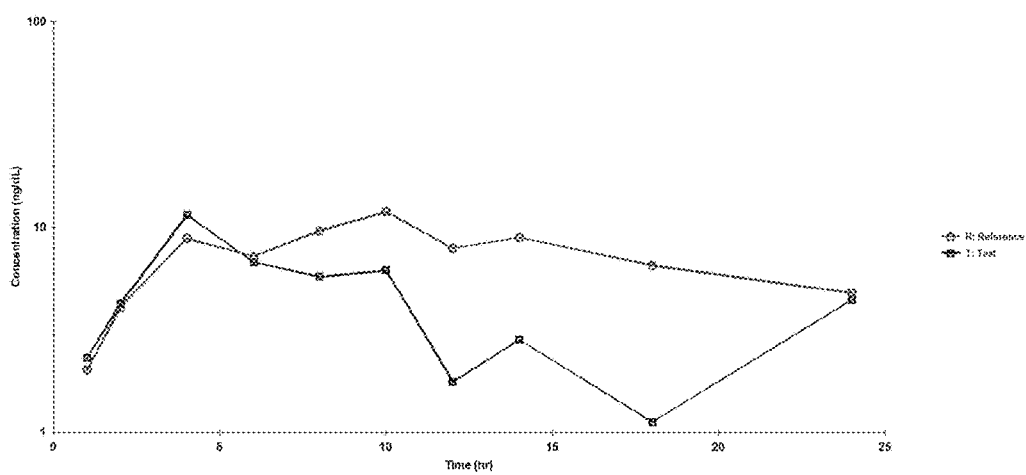


FIG. 14

VAGINAL INSERTED ESTRADIOL PHARMACEUTICAL COMPOSITIONS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 14/521,230, filed Oct. 22, 2014, which claims priority to U.S. Provisional Patent Application Nos. 61/894,411, filed Oct. 22, 2013, and 61/932,140, filed Jan. 27, 2014, and which is a continuation-in-part of International Patent Application No. PCT/US2013/046443, filed Jun. 18, 2013, which claims priority to U.S. Provisional Patent Application No. 61/745,313, filed Dec. 21, 2012. All aforementioned applications are hereby incorporated by reference herein in their entirety.

BACKGROUND

This application is directed to pharmaceutical compositions, methods, and devices related to hormone replacement therapy.

Postmenopausal women frequently suffer from atrophic vaginitis or vulvar and vaginal atrophy (hereinafter “vulvovaginal atrophy” or “VVA”) with symptoms including, for example, vaginal dryness, vaginal odor, vaginal or vulvar irritation or itching, dysuria (pain, burning, or stinging when urinating), dyspareunia (vaginal pain associated with sexual activity), or vaginal bleeding associated with sexual activity. Other symptoms include soreness; with urinary frequency and urgency; urinary discomfort and incontinence also occurring (“estrogen-deficient urinary state(s)”). One symptom of vaginal atrophy is an increased vaginal pH, which creates an environment more susceptible to infections. The mucosal epithelium of the VVA patients also reported to show signs of severe atrophy and upon cytological examination accompanied by an increased number of the parabasal cells and a reduced number of superficial cells.

Each of these VVA-related states manifest symptoms associated with decreased estrogenization of the vulvovaginal tissue, and can even occur in women treated with oral administration of an estrogen-based pharmaceutical drug product. Although VVA is most common with menopausal women, it can occur at any time in a woman's life cycle.

Estrogen treatment has proven to be very successful in controlling menopausal symptoms, including vaginal atrophy (VVA). Several studies have shown that the symptoms connected with vaginal atrophy are often relieved by estrogen treatment given either systemically or topically. The existing treatments have numerous problems, for example compliance issues with patients not completing or continuing treatment due to the problems associated with the form of treatment.

Accordingly, disclosed herein is, among other things, a new soft gel vaginal pharmaceutical composition and dosage form containing solubilized estradiol for the treatment of VVA. The soft gel vaginal pharmaceutical composition has been designed to mitigate common limitations found with other vaginal forms of estradiol. The soft gel vaginal pharmaceutical composition is expected to ease vaginal administration, provide improved safety of insertion, minimize vaginal discharge following administration, and provide a more effective dosage form with improved efficacy, safety and patient compliance.

SUMMARY

According to various aspects and embodiments of this disclosure, a soft gel vaginal pharmaceutical composition as

a potential treatment for post-menopausal women suffering with moderate to severe symptoms of VVA is provided.

Provided herein is a pessary comprising: a) a therapeutically effective amount of estradiol; and b) a solubilizing agent comprising a medium chain oil.

In some embodiments, the pessary comprises about 1 µg to about 25 µg of estradiol. For example, the pessary can include about 1 µg to about 10 µg of estradiol; and about 10 µg to about 25 µg of estradiol.

In some embodiments, the estradiol is solubilized.

In some embodiments, the medium chain oil comprises at least one C6-C12 fatty acid or a glycol, monoglyceride, diglyceride, or triglyceride ester thereof.

In some embodiments, the solubilizing agent comprises at least one ester selected from the group consisting of: an ester of capric fatty acid, an ester of caprylic fatty acid, an ester of capric fatty acid, and combinations thereof. For example, the solubilizing agent can include a caprylic/capric triglyceride.

In some embodiments, the pessary further comprises a capsule. For example, the capsule can be a soft gelatin capsule.

Also provided herein is a pessary comprising: a) a therapeutically effective amount of estradiol; b) a caprylic/capric triglyceride; c) a non-ionic surfactant comprising PEG-6 stearate and ethylene glycol palmitostearate; and d) a soft gelatin capsule.

In some embodiments, a pessary provided herein comprises about 25 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 19 pg/ml to about 29 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 75 pg*hr/ml to about 112 pg*hr/ml.

In some embodiments, a pessary provided herein comprises about 25 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 9 pg/ml to about 14 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 43 pg*hr/ml to about 65 pg*hr/ml.

In some embodiments, a pessary provided herein comprises about 25 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 416 pg/ml to about 613 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 3598 pg*hr/ml to about 5291 pg*hr/ml.

In some embodiments, a pessary provided herein comprises about 10 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 12 pg/ml to about 18 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 42 pg*hr/ml to about 63 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estradiol of about 1 hrs to about 3 hrs.

In some embodiments, a pessary provided herein comprises about 10 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 4 pg/ml to about 7

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pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 20 pg*hr/ml to about 31 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone of about 4 hrs to about 8 hrs.

In some embodiments, a pessary provided herein comprises about 10 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 10 pg/ml to about 16 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 56 pg*hr/ml to about 84 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone sulfate of about 4 hrs to about 7 hrs.

In some embodiments, a pessary provided herein comprises about 4 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 4 pg/ml to about 8 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 16 pg*hr/ml to about 26 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estradiol of about 0.25 hrs to about 2 hrs.

In some embodiments, a pessary provided herein comprises about 4 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 1 pg/ml to about 3 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 8 pg*hr/ml to about 13 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone of about 1 hrs to about 4 hrs.

In some embodiments, a pessary provided herein comprises about 4 µg of estradiol, wherein administration of the pessary to a patient provides, in a plasma sample from the patient: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 4 pg/ml to about 7 pg/ml; and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 22 pg*hr/ml to about 34 pg*hr/ml. In some embodiments, the pessary further provides a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone sulfate of about 1 hrs to about 3 hrs.

Also provided herein is a pessary comprising about 1 µg to about 25 µg of estradiol, wherein administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estradiol that is less than about 30 pg/ml. For example, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estradiol that is less than about 18 pg/ml.

In some embodiments, a pessary comprising about 1 µg to about 25 µg of estradiol is provided, wherein administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol that is less than about 112 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol that is less than about 63 pg*hr/ml.

In some embodiments, a pessary comprising about 1 µg to about 25 µg of estradiol is provided, wherein administration of the pessary to a patient provides a corrected geometric

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mean peak plasma concentration (C_{max}) of estrone that is less than about 14 pg/ml. For example, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone that is less than about 7 pg/ml.

In some embodiments, a pessary comprising about 1 µg to about 25 µg of estradiol is provided, wherein administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone that is less than about 65 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone that is less than about 31 pg*hr/ml.

In some embodiments, a pessary comprising about 1 µg to about 25 µg of estradiol is provided, wherein administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate that is less than about 613 pg/ml. For example, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate that is less than about 16 pg/ml.

In some embodiments, a pessary comprising about 1 µg to about 25 µg of estradiol is provided, wherein administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate that is less than about 5291 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate that is less than about 84 pg*hr/ml.

Further provided herein is a pessary comprising about 1 µg to about 25 µg of estradiol, wherein administration of the pessary to the proximal region of the vagina of a patient provides a therapeutically effective concentration of estradiol over 24 hours in the proximal region of the vagina.

This disclosure also provides a method of treating an estrogen-deficient state, the method comprising administering to a patient in need thereof, a pessary as provided herein. In some embodiments, a method of treating vulvovaginal atrophy is provided, the method comprising administering to a patient in need thereof, a pessary as provided herein.

In some embodiments of the methods provided herein, treatment comprises reducing the severity of one or more symptoms selected from the group consisting of: vaginal dryness, dyspareunia, vaginal or vulvar irritation, vaginal or vulvar burning, vaginal or vulvar itching, dysuria, and vaginal bleeding associated with sexual activity.

In some embodiments of the methods provided herein treatment comprises reducing the vaginal pH of the patient. For example, treatment comprises reducing the vaginal pH of the patient to a pH of less than about 5.0.

In some embodiments of the methods provided herein treatment comprises a change in cell composition of the patient. For example, the change in cell composition comprises reducing the number of parabasal vaginal cells or increasing the number of superficial vaginal cells. In some embodiments, the number of parabasal vaginal cells in the patient are reduced by at least about 35% (e.g., at least about 50%). In some embodiments, the number of superficial vaginal cells are increased by at least about 5% (e.g., at least about 35%).

Further provided herein is a method for reducing vaginal discharge following administration of a pessary, the method comprising administering to a patient in need thereof, a pessary provided herein, wherein the vaginal discharge following administration of the pessary is compared to the vaginal discharge following administration of a reference drug.

The above-mentioned features and objects of the this disclosure will become more apparent with reference to the following description taken in conjunction with the accompanying drawings wherein like reference numerals denote like elements and in which:

FIG. 1 is a flow diagram illustrating a process in accordance with various embodiments of the invention;

FIG. 2 illustrates a suppository in accordance with various embodiments of the invention;

FIG. 3 is a linear plot of mean plasma estradiol-baseline adjusted concentrations versus time (N=36);

FIG. 4 is a semi-logarithmic plot of mean plasma estradiol-baseline adjusted concentrations versus time (N=36);

FIG. 5 is a linear plot of mean plasma estrone-baseline adjusted concentrations versus time (N=36);

FIG. 6 is a semi-logarithmic plot of mean plasma estrone-baseline adjusted concentrations versus time (N=36);

FIG. 7 is a linear plot of mean plasma estrone sulfate-baseline adjusted concentrations versus time (N=36);

FIG. 8 is a semi-logarithmic plot of mean plasma estrone sulfate-baseline adjusted concentrations versus time (N=36);

FIG. 9 is a linear plot of mean plasma estradiol-baseline adjusted concentrations versus time (N=34);

FIG. 10 is a semi-logarithmic plot of mean plasma estradiol-baseline adjusted concentrations versus time (N=34);

FIG. 11 is a linear plot of mean plasma estrone-baseline adjusted concentrations versus time (N=33);

FIG. 12 is a semi-logarithmic plot of mean plasma estrone-baseline adjusted concentrations versus time (N=33);

FIG. 13 is a linear plot of mean plasma estrone sulfate-baseline adjusted concentrations versus time (N=24); and

FIG. 14 is a semi-logarithmic plot of mean plasma estrone sulfate-baseline adjusted concentrations versus time (N=24).

DETAILED DESCRIPTION

In the following detailed description of embodiments of this disclosure, reference is made to the accompanying drawings in which like references indicate similar elements, and in which is shown by way of illustration specific embodiments in which the this disclosure may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the this disclosure, and it is to be understood that other embodiments may be utilized and that other changes may be made without departing from the scope of the this disclosure. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of this disclosure is defined only by the appended claims. As used in this disclosure, the term “or” shall be understood to be defined as a logical disjunction (i.e., and/or) and shall not indicate an exclusive disjunction unless expressly indicated as such with the terms “either,” “unless,” “alternatively,” and words of similar effect.

Definitions

The term “active pharmaceutical ingredient” (“API”) as used herein, means the active compound(s) used in formulating a drug product.

The term “co-administered” as used herein, means that two or more drug products are administered simultaneously or sequentially on the same or different days.

The term “drug product” as used herein means at least one active pharmaceutical ingredient in combination with at least one excipient and provided in unit dosage form.

The term “area under the curve” (“AUC”) refers to the area under the curve defined by changes in the blood concentration of an active pharmaceutical ingredient (e.g., estradiol or progesterone), or a metabolite of the active pharmaceutical ingredient, over time following the administration of a dose of the active pharmaceutical ingredient. “AUC_{0-∞}” is the area under the concentration-time curve extrapolated to infinity following the administration of a dose. “AUC_{0-t}” is the area under the concentration-time curve from time zero to time t following the administration of a dose, wherein t is the last time point with a measurable concentration.

The term “C_{max}” refers to the maximum value of blood concentration shown on the curve that represents changes in blood concentrations of an active pharmaceutical ingredient (e.g., progesterone or estradiol), or a metabolite of the active pharmaceutical ingredient, over time.

The term “T_{max}” refers to the time that it takes for the blood concentration an active pharmaceutical ingredient (e.g., estradiol or progesterone), or a metabolite of the active pharmaceutical ingredient, to reach the maximum value.

The term “bioavailability,” which has the meaning defined in 21 C.F.R. § 320.1(a), refers to the rate and extent to which an API or active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For example, bioavailability can be measured as the amount of API in the blood (serum or plasma) as a function of time. Pharmacokinetic (PK) parameters such as AUC, C_{max}, or T_{max} may be used to measure and assess bioavailability. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the API or active ingredient or active moiety becomes available at the site of action.

The term “bioequivalent,” which has the meaning defined in 21 C.F.R. § 320.1(e), refers to the absence of a significant difference in the rate and extent to which the API or active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Where there is an intentional difference in rate (e.g., in certain extended release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug. In practice, two products are considered bioequivalent if the 90% confidence interval of the AUC, C_{max}, or optionally T_{max} is within 80.00% to 125.00%.

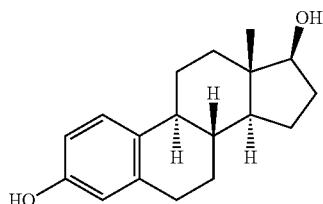
The term “bio-identical,” “body-identical,” or “natural” used in conjunction with the hormones disclosed herein, means hormones that match the chemical structure and effect of those that occur naturally or endogenously in the human body. An exemplary natural estrogen is estradiol.

The term “bio-identical hormone” or “body-identical hormone” refers to an active pharmaceutical ingredient that is

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structurally identical to a hormone naturally or endogenously found in the human body (e.g., estradiol and progesterone).

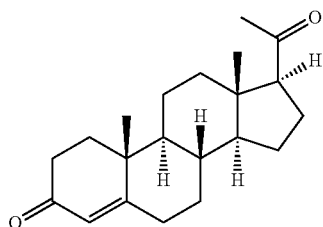
The term “estradiol” refers to (17 β)-estra-1,3,5(10)-triene-3,17-diol. Estradiol is also interchangeably called 17 β -estradiol, oestradiol, or E2, and is found endogenously in the human body. As used herein, estradiol refers to the bio-identical or body-identical form of estradiol found in the human body having the structure:



Estradiol is supplied in an anhydrous or hemi-hydrate form. For the purposes of this disclosure, the anhydrous form or the hemihydrate form can be substituted for the other by accounting for the water or lack of water according to well-known and understood techniques.

The term “solubilized estradiol” means that the estradiol or a portion thereof is solubilized or dissolved in the solubilizing agent(s) or the formulations disclosed herein. Solubilized estradiol may include estradiol that is about 80% solubilized, about 85% solubilized, about 90% solubilized, about 95% solubilized, about 96% solubilized, about 97% solubilized, about 98% solubilized, about 99% solubilized or about 100% solubilized. In some embodiments, the estradiol is “fully solubilized” with all or substantially all of the estradiol being solubilized or dissolved in the solubilizing agent. Fully solubilized estradiol may include estradiol that is about 97% solubilized, about 98% solubilized, about 99% solubilized or about 100% solubilized. Solubility can be expressed as a mass fraction (% w/w, which is also referred to as wt %).

The term “progesterone” refers to pregn-4-ene-3,20-dione. Progesterone is also interchangeably called P₄ and is found endogenously in the human body. As used herein, progesterone refers to the bio-identical or body-identical form of progesterone found in the human body having the structure:



The term “solubilized progesterone” means that the progesterone or a portion thereof is solubilized or dissolved in the solubilizing agent(s) or the formulations disclosed herein. In some embodiments, the progesterone is “partially solubilized” with a portion of the progesterone being solubilized or dissolved in the solubilizing agent and a portion of the progesterone being suspended in the solubilizing agent. Partially solubilized progesterone may include progesterone that is about 1% solubilized, about 5% solubilized, about

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10% solubilized, about 15% solubilized, about 20% solubilized, about 30% solubilized, about 40% solubilized, about 50% solubilized, about 60% solubilized, about 70% solubilized, about 80% solubilized, about 85% solubilized, about 90% solubilized or about 95% solubilized. In other embodiments, the progesterone is “fully solubilized” with all or substantially all of the progesterone being solubilized or dissolved in the solubilizing agent. Fully solubilized progesterone may include progesterone that is about 97% solubilized, about 98% solubilized, about 99% solubilized or about 100% solubilized. Solubility can be expressed as a mass fraction (% w/w, which is also referred to as wt %).

The terms “micronized progesterone” and “micronized estradiol,” as used herein, include micronized progesterone and micronized estradiol having an X50 particle size value below about 15 microns or having an X90 particle size value below about 25 microns. The term “X50” means that one-half of the particles in a sample are smaller in diameter than a given number. For example, micronized progesterone having an X50 of 5 microns means that, for a given sample of micronized progesterone, one-half of the particles have a diameter of less than 5 microns. Similarly, the term “X90” means that ninety percent (90%) of the particles in a sample are smaller in diameter than a given number.

The term “glyceride” is an ester of glycerol (1,2,3-propanetriol) with acyl radicals of fatty acids and is also known as an acylglycerol. If only one position of the glycerol molecule is esterified with a fatty acid, a “mono-glyceride” or “monoacylglycerol” is produced; if two positions are esterified, a “diglyceride” or “diacylglycerol” is produced; and if all three positions of the glycerol are esterified with fatty acids, a “triglyceride” or “triacylglycerol” is produced. A glyceride is “simple” if all esterified positions contain the same fatty acid; whereas a glyceride is “mixed” if the esterified positions contained different fatty acids. The carbons of the glycerol backbone are designated sn-1, sn-2 and sn-3, with sn-2 being in the middle carbon and sn-1 and sn-3 being the end carbons of the glycerol backbone.

The term “solubilizing agent” refers to an agent or combination of agents that solubilize an active pharmaceutical ingredient (e.g., estradiol or progesterone). For example and without limitation, suitable solubilizing agents include medium chain oils and other solvents and co-solvents that solubilize or dissolve an active pharmaceutical ingredient to a desirable extent. Solubilizing agents suitable for use in the formulations disclosed herein are pharmaceutical grade solubilizing agents (e.g., pharmaceutical grade medium chain oils). It will be understood by those of skill in the art that other excipients or components can be added to or mixed with the solubilizing agent to enhance the properties or performance of the solubilizing agent or resulting formulation. Examples of such excipients include, but are not limited to, surfactants, emulsifiers, thickeners, colorants, flavoring agents, etc. In some embodiments, the solubilizing agent is a medium chain oil and, in some other embodiments, the medium chain oil is combined with a co-solvent(s) or other excipient(s).

The term “medium chain” is used to describe the aliphatic chain length of fatty acid containing molecules. “Medium chain” specifically refers to fatty acids, fatty acid esters, or fatty acid derivatives that contain fatty acid aliphatic tails or carbon chains that contain 6 (C6) to 14 (C14) carbon atoms, 8 (C8) to 12 (C12) carbon atoms, or 8 (C8) to 10 (C10) carbon atoms.

The terms “medium chain fatty acid” and “medium chain fatty acid derivative” are used to describe fatty acids or fatty

acid derivatives with aliphatic tails (i.e., carbon chains) having 6 to 14 carbon atoms. Fatty acids consist of an unbranched or branched aliphatic tail attached to a carboxylic acid functional group. Fatty acid derivatives include, for example, fatty acid esters and fatty acid containing molecules, including, without limitation, mono-, di- and triglycerides that include components derived from fatty acids. Fatty acid derivatives also include fatty acid esters of ethylene or propylene glycol. The aliphatic tails can be saturated or unsaturated (i.e., having one or more double bonds between carbon atoms). In some embodiments, the aliphatic tails are saturated (i.e., no double bonds between carbon atoms). Medium chain fatty acids or medium chain fatty acid derivatives include those with aliphatic tails having 6-14 carbons, including those that are C6-C14, C6-C12, C8-C14, C8-C12, C6-C10, C8-C10, or others. Examples of medium chain fatty acids include, without limitation, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, and derivatives thereof.

The term "oil," as used herein, refers to any pharmaceutically acceptable oil, especially medium chain oils, and specifically excluding peanut oil, that can suspend or solubilize bioidentical progesterone or estradiol, including starting materials or precursors thereof, including micronized progesterone or micronized estradiol as described herein.

The term "medium chain oil" refers to an oil wherein the composition of the fatty acid fraction of the oil is substantially medium chain (i.e., C6 to C14) fatty acids, i.e., the composition profile of fatty acids in the oil is substantially medium chain. As used herein, "substantially" means that between 20% and 100% (inclusive of the upper and lower limits) of the fatty acid fraction of the oil is made up of medium chain fatty acids, i.e., fatty acids with aliphatic tails (i.e., carbon chains) having 6 to 14 carbons. In some embodiments, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 85%, about 90% or about 95% of the fatty acid fraction of the oil is made up of medium chain fatty acids. Those of skill in the art that will readily appreciate that the terms "alkyl content" or "alkyl distribution" of an oil can be used in place of the term "fatty acid fraction" of an oil in characterizing a given oil or solubilizing agent, and these terms are used interchangeable herein. As such, medium chain oils suitable for use in the formulations disclosed herein include medium chain oils wherein the fatty acid fraction of the oil is substantially medium chain fatty acids, or medium chain oils wherein the alkyl content or alkyl distribution of the oil is substantially medium chain alkyls (C6-C12 alkyls). It will be understood by those of skill in the art that the medium chain oils suitable for use in the formulations disclosed herein are pharmaceutical grade (e.g., pharmaceutical grade medium chain oils). Examples of medium chain oils include, for example and without limitation, medium chain fatty acids, medium chain fatty acid esters of glycerol (e.g., for example, mono-, di-, and triglycerides), medium chain fatty acid esters of propylene glycol, medium chain fatty acid derivatives of polyethylene glycol, and combinations thereof.

The term "ECN" or "equivalent carbon number" means the sum of the number of carbon atoms in the fatty acid chains of an oil, and can be used to characterize an oil as, for example, a medium chain oil or a long-chain oil. For example, tripalmitin (tripalmitic glycerol), which is a simple triglyceride containing three fatty acid chains of 16 carbon atoms, has an ECN of $3 \times 16 = 48$. Conversely, a triglyceride with an ECN=40 may have "mixed" fatty acid chain lengths of 8, 16 and 16; 10, 14 and 16; 8, 14 and 18; etc. Naturally

occurring oils are frequently "mixed" with respect to specific fatty acids, but tend not to contain both long chain fatty acids and medium chain fatty acids in the same glycerol backbone. Thus, triglycerides with ECN's of 21-42 typically contain predominately medium chain fatty acids; while triglycerides with ECN's of greater than 43 typically contain predominantly long chain fatty acids. For example, the ECN of corn oil triglyceride in the USP would be in the range of 51-54. Medium chain diglycerides with ECN's of 12-28 will often contain predominately medium chain fatty chains, while diglycerides with ECN's of 32 or greater will typically contain predominately long chain fatty acid tails. Monoglycerides will have an ECN that matches the chain length of the sole fatty acid chain. Thus, monoglyceride ECN's in the range of 6-14 contain mainly medium chain fatty acids, and monoglycerides with ECN's 16 or greater will contain mainly long chain fatty acids.

The average ECN of a medium chain triglyceride oil is typically 21-42. For example, as listed in the US Pharmacopeia (USP), medium chain triglycerides have the following composition as the exemplary oil set forth in the table below:

Fatty-acid Tail Length	% of oil	Exemplary Oil
6	≤ 2.0	2.0
8	50.0-80.0	70.0
10	20.0-50.0	25.0
12	≤ 3.0	2.0
14	≤ 1.0	1.0

and would have an average ECN of $3 * [(6 * 0.02) + (8 * 0.70) + (10 * 0.25) + (12 * 0.02) + (14 * 0.01)] = 25.8$. The ECN of the exemplary medium chain triglycerides oil can also be expressed as a range (per the ranges set forth in the USP) of 24.9-27.0. For oils that have mixed mono-, di-, and triglycerides, or single and double fatty acid glycols, the ECN of the entire oil can be determined by calculating the ECN of each individual component (e.g., C8 monoglycerics, C8 diglycerides, C10 monoglycerides, and C10 monoglycerides) and taking the sum of the relative percentage of the component multiplied by the ECN normalized to a monoglyceride for each component. For example, the oil having C8 and C10 mono- and diglycerides shown in the table below has an ECN of 8.3, and is thus a medium chain oil.

Fatty-acid Chain Length	ECN as % of oil (chain length) \times (% in % of oil oil)	ECN as % of oil normalized to monoglyceride
C8 monoglyceride	$47 \quad 8 \times 0.47 = 3.76$	3.76
C10 monoglyceride	$8 \quad 10 \times 0.08 = 0.8$	0.8
C8 diglyceride	$38 \quad 2 \times (8 \times 0.38) = 6.08$	$6.08/2 = 3.04$
C10 diglyceride	$7 \quad 2 \times (10 \times 0.07) = 1.4$	$1.4/2 = 0.7$
OIL ECN (normalized to monoglycerides)		8.3

Expressed differently, ECN can be calculated as each chain length in the composition multiplied by its relative percentage in the oil: $(8 * 0.85) + (10 * 0.15) = 8.3$.

The term "excipients," as used herein, refers to non-API ingredients such as solubilizing agents, anti-oxidants, oils, lubricants, and others used in formulating pharmaceutical products.

The term "patient" or "subject" refers to an individual to whom the pharmaceutical composition is administered.

The term “pharmaceutical composition” refers to a pharmaceutical composition comprising at least a solubilizing agent and estradiol. As used herein, pharmaceutical compositions are delivered, for example via pessary (i.e., vaginal suppository), or absorbed vaginally.

The term “progestin” means any natural or man-made substance that has pharmacological properties similar to progesterone.

The term “reference listed drug product” (“RLD”) means VAGIFEM® (estradiol vaginal tablets) or ESTRACE® vaginal cream.

The terms “treat,” “treating,” and “treatment” refer to any indicia of success in the treatment or amelioration of an injury, disease, or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, disease, or condition more tolerable to the patient; slowing in the rate of degeneration or decline; or improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subject parameters, including the results of a physical examination, neuropsychiatric examinations, or psychiatric evaluation.

The terms “atrophic vaginitis,” “vulvovaginal atrophy,” “vaginal atrophy,” and “VVA” are used herein interchangeably. The molecular morphology of VVA is well known in the medical field.

Introduction

Provided herein are pharmaceutical compositions comprising solubilized estradiol designed to be absorbed vaginally. The pharmaceutical compositions disclosed herein are designed to be absorbed and have their therapeutic effect locally, e.g., in vaginal or surrounding tissue. Further disclosed herein are data demonstrating efficacy of the pharmaceutical compositions disclosed, as well as methods relating to the pharmaceutical compositions. Generally, the pharmaceutical compositions disclosed herein are useful in VVA, dyspareunia, and other indications caused by decrease or lack of estrogen.

Additional aspects and embodiments of this disclosure include: providing increased patient ease of use while potentially minimizing certain side effects from inappropriate insertion, minimizing incidence of vulvovaginal mycotic infection compared to incidence of vulvovaginal mycotic infection due to usage of other vaginally applied estradiol products; and, improved side effect profile (e.g., pruritus) compared to the reference drug: VAGIFEM® (estradiol vaginal tablets, Novo Nordisk; Princeton, N.J.).

Pharmaceutical Composition

Functionality

According to embodiments, the pharmaceutical compositions disclosed herein are alcohol-free or substantially alcohol-free. The pharmaceutical compositions offer provide for improved patient compliance because of improvements over the prior offering. According to embodiments, the pharmaceutical compositions disclosed herein are encapsulated in soft gelatin capsules, which improve comfort during use. According to embodiments, the pharmaceutical compositions are substantially liquid, which are more readily absorbed in the vaginal tissue, and also are dispersed over a larger surface area of the vaginal tissue.

Estradiol

According to embodiments, the pharmaceutical compositions disclosed herein are for vaginal insertion in a single

or multiple unit dosage form. According to embodiments, the estradiol in the pharmaceutical compositions is at least about: 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% solubilized. According to embodiments and where the estradiol is not 100% solubilized, the remaining estradiol is present in a micronized (crystalline) form that is absorbable by the body and retains biological functionality, either in its micronized form or in another form which the micronized form is converted to after administration.

According to embodiments, all or some of the estradiol is solubilized in a solubilizing agent during manufacturing process. According to embodiments, all or some of the estradiol is solubilized following administration (e.g., the micronized portion where the estradiol is not 100% solubilized is solubilized in a body fluid after administration). According to embodiments, because the estradiol is solubilized, the solubilizing agents taught herein, with or without additional excipients other than the solubilizing agents, are liquid or semi-solid. To the extent the estradiol is not fully solubilized at the time of administration/insertion, the estradiol should be substantially solubilized at a body temperature (average of 37° C.) and, generally, at the pH of the vagina (ranges from 3.8 to 4.5 in healthy patients; and 4.6 to 6.5 in VVA patients).

According to embodiments, the estradiol can be added to the pharmaceutical compositions disclosed herein as estradiol, estradiol hemihydrate, or other grade estradiol forms used in pharmaceutical compositions or formulations.

According to embodiments, estradiol dosage strengths vary. Estradiol (or estradiol hemihydrate, for example, to the extent the water content of the estradiol hemihydrate is accounted for) dosage strength of is from at least about 1 microgram (μg or μg) to at least about 50 μg . Specific dosage embodiments contain at least about: 1 μg , 2 μg , 3 μg , 4 μg , 5 μg , 6 μg , 7 μg , 8 μg , 9 μg , 10 μg , 11 μg , 12 μg , 13 μg , 14 μg , 15 μg , 16 μg , 17 μg , 18 μg , 19 μg , 20 μg , 21 μg , 22 μg , 23 μg , 24 μg , 25 μg , 26 μg , 27 μg , 28 μg , 29 μg , 30 μg , 31 μg , 32 μg , 33 μg , 34 μg , 35 μg , 36 μg , 37 μg , 38 μg , 39 μg , 40 μg , 41 μg , 42 μg , 43 μg , 44 μg , 45 μg , 46 μg , 47 μg , 48 μg , 49 μg , or 50 μg estradiol. According to embodiments, the pharmaceutical compositions contain at least about 2.5 μg ; 4 μg 6.25 μg , 7.5 μg , 12.5 μg , 18.75 μg of estradiol. According to embodiments, the pharmaceutical compositions contain from about 1 μg to about 10 μg , from 3 μg to 7 μg , from about 7.5 μg to 12.5 μg , from about 10 μg to about 25 μg , about 1 μg , about 2.5 μg , from about 23.5 μg to 27.5 μg , from about 7.5 μg to 22.5 μg , from 10 μg to 25 μg of estradiol. The lowest clinically effective dose of estradiol is used for treatment of VVA and other indications set forth herein. In some embodiments, the estradiol dosage is about 4 μg . In one embodiment, the estradiol dosage is about 10 μg . In another embodiment, the estradiol dosage is about 25 μg .

Solvent System

According to embodiments, the solvent system that solubilizes the estradiol are medium chain fatty acid based solvents, together with other excipients. According to embodiments, the solvent system comprises non-toxic, pharmaceutically acceptable solvents, co-solvents, surfactants, and other excipients suitable for vaginal delivery or absorption.

According to embodiments, oils having medium chain fatty acids as a majority component are used as solubilizing agents to solubilize estradiol. According to embodiments, the solubilizing agents comprise medium chain fatty acid

esters (e.g., esters of glycerol, ethylene glycol, or propylene glycol) or mixtures thereof. According to embodiments, the medium chain fatty acids comprise chain lengths from C6 to C14. According to embodiments the medium chain fatty acids comprise chain lengths from C6 to C12. According to

embodiments the medium chain fatty acids substantially comprise chain lengths from C8-C10. ECN's for medium chain oils will be in the range of 21-42 for triglycerides, 12-28 for diglycerides, and 6-14 for monoglycerides.

According to embodiments, the medium chain fatty acids are saturated. According to embodiments, the medium chain fatty acids are predominantly saturated, i.e., greater than about 60% or greater than about 75% saturated.

According to embodiments, estradiol is soluble in the solubilizing agent at room temperature, although it may be desirable to warm certain solubilizing agents during manufacture to improve viscosity. According to embodiments, the solubilizing agent is liquid at between room temperature and about 50° C., at or below 50° C., at or below 40° C., or at or below 30° C.

According to embodiments, the solubility of estradiol in the medium chain oil, medium chain fatty acid, or solubilizing agent (or oil/surfactant) is at least about 0.01 wt %, 0.02 wt %, 0.05 wt %, 0.06 wt %, 0.08 wt %, 0.1 wt %, 0.2 wt %, 0.3 wt %, 0.4 wt %, 0.5 wt %, 0.6 wt %, 0.7 wt %, 0.8 wt %, 0.9 wt %, 1.0 wt %, or higher.

According to embodiments, medium chain solubilizing agents include, for example and without limitation saturated medium chain fatty acids: caproic acid (C6), enanthic acid (C7), caprylic acid (C8), pelargonic acid (C9), capric acid (C10), undecylic acid (C11), lauric acid (C12), tridecylic acid (C13), or myristic acid (C14). According to embodiments, the solubilizing agent comprises oils made of these free medium chain fatty acids, oils of medium chain fatty acid esters of glycerin, propylene glycol, or ethylene glycol, or combinations thereof. These examples comprise predominantly saturated medium chain fatty acids (i.e., greater than 50% of the fatty acids are medium chain saturated fatty acids). According to embodiments, predominantly C6 to C12 saturated fatty acids are contemplated. According to embodiments, the solubilizing agent is selected from at least one of a solvent or co-solvent.

According to embodiments, glycerin based solubilizing agents include: mono-, di-, or triglycerides and combinations and derivatives thereof. Exemplary glycerin based solubilizing agents include MIGLYOLs®, which are caprylic/capric triglycerides (SASOL Germany GMBH, Hamburg). MIGLYOLs includes MIGLYOL Bio (caprylic/capric triglyceride), MIGLYOL 812 (caprylic/capric triglyceride), MIGLYOL 816 (caprylic/capric triglyceride), and MIGLYOL 829 (caprylic/capric/succinic triglyceride). Other caprylic/capric triglyceride solubilizing agents are likewise contemplated, including, for example: caproic/caprylic/capric/lauric triglycerides; caprylic/capric/linoleic triglycerides; caprylic/capric/succinic triglycerides. According to embodiments, CAPMUL MCM, medium chain mono- and di-glycerides, is the solubilizing agent. Other and triglycerides of fractionated vegetable fatty acids, and combinations or derivatives thereof can be the solubilizing agent, according to embodiments. For example, the solubilizing agent can be 1,2,3-propanetriol (glycerol, glycerin, glycerine) esters of saturated coconut and palm kernel oil and derivatives thereof.

Ethylene and propylene glycols (which include polyethylene and polypropylene glycols) solubilizing agents include: glyceryl mono- and di-caprylates; propylene glycol monocaprylate (e.g., CAPMUL® PG-8 (the CAPMUL

brands are owned by ABITEC, Columbus, Ohio)); propylene glycol monocaprate (e.g., CAPMUL PG-10); propylene glycol mono- and dicaprylates; propylene glycol mono- and dicaprate; diethylene glycol mono ester (e.g., TRANSCUTOL®, 2-(2-Ethoxyethoxy)ethanol, GATTEFOSSÉ SAS); and diethylene glycol monoethyl ether. Other combinations of mono- and di-esters of propylene glycol or ethylene glycol are expressly contemplated are the solubilizing agent.

According to embodiments, the solubilizing agent comprises combinations of mono- and di-propylene and ethylene glycols and mono-, di-, and triglyceride combinations. According to embodiments, polyethylene glycol glyceride (GELUCIRE®, GATTEFOSSÉ, SAS, Saint-Priest, France) can be used herein as the solubilizing agent or as a surfactant. For example, GELUCIRE 44/14 (PEG-32 glyceryl laurate EP), a medium chain fatty acid esters of polyethylene glycol, is a polyethylene glycol glyceride composed of mono-, di- and triglycerides and mono- and diesters of polyethylene glycol.

According to embodiments, commercially available fatty acid glycerol and glycol ester solubilizing agents are often prepared from natural oils and therefore may comprise components in addition to the fatty acid esters that predominantly comprise and characterize the solubilizing agent. Such other components may be, e.g., other fatty acid mono-, di-, and triglycerides; fatty acid mono- and diester ethylene or propylene glycols, free glycerols or glycols, or free fatty acids, for example. In some embodiments, when an oil/solubilizing agent is described herein as a saturated C₈ fatty acid mono- or diester of glycerol, the predominant component of the oil, i.e., >50 wt % (e.g., >75 wt %, >85 wt % or >90 wt %) is caprylic monoglycerides and caprylic diglycerides. For example, the Technical Data Sheet by ABITEC for CAPMUL MCM C8 describes CAPMUL MCM C8 as being composed of mono and diglycerides of medium chain fatty acids (mainly caprylic) and describes the alkyl content as 1% C6, ≥95% C8, ≤5% C10, and ≤1.5% C12 and higher.

For example, MIGLYOL 812 is a solubilizing agent that is generally described as a C8-C10 triglyceride because the fatty acid composition is at least about 80% triglyceride esters of caprylic acid (C8) and capric acid (C10). However, it also comprises small amounts of other fatty acids, e.g., less than about 5% of caproic acid (C6), lauric acid (C12), and myristic acid (C14). The product information sheet for various MIGLYOLs illustrate the various fatty acid components as follows:

	Tests				
	810	812	818	829	840
Caproic acid (C6:0)	max. 2.0	max. 2.0	max. 2	max. 2	max. 2
Caprylic acid (C8:0)	65.0-80.0	50.0-65.0	45-65	45-55	65-80
Capric acid (C10:0)	20.0-35.0	30.0-45.0	30-45	30-40	20-35
Lauric acid (C12:0)	max. 2	max. 2	max. 3	max. 3	max. 2
Myristic acid (C14:0)	max. 1.0	max. 1.0	max. 1	max. 1	max. 1
Linoleic acid (C18:2)	—	—	2-5	—	—

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-continued

	Tests				
	810	812	818	829	840
Succinic acid	—	—	—	15-20	—
ECN	25.5-26.4	26.1-27	26.52-28.56	26-27.6	25.5-26.4

According to embodiments, anionic or non-ionic surfactants may be used in pharmaceutical compositions containing solubilized estradiol. Ratios of solubilizing agent(s) to surfactant(s) vary depending upon the respective solubilizing agent(s) and the respective surfactant(s) and the desired physical characteristics of the resultant pharmaceutical composition. For example and without limitation, CAPMUL MCM and a non-ionic surfactant may be used at ratios including 65:35, 70:30, 75:25, 80:20, 85:15 and 90:10. Other non-limiting examples include: CAPMUL MCM and GELUCIRE 39/01 used in ratios including, for example and without limitation, 6:4, 7:3, and 8:2; CAPMUL MCM and GELUCIRE 43/01 used in ratios including, for example and without limitation, 7:3, and 8:2; CAPMUL MCM and GELUCIRE 50/13 used in ratios including, for example and without limitation, 7:3, and 8:2, and 9:1.

Other Excipients

According to embodiments, the pharmaceutical composition further comprises a surfactant. The surfactant can be a nonionic surfactant, cationic surfactant, anionic surfactant, or mixtures thereof. Suitable surfactants include, for example, water-insoluble surfactants having a hydrophilic-lipophilic balance (HLB) value less than 12 and water-soluble surfactants having a HLB value greater than 12. Surfactants that have a high HLB and hydrophilicity, aid the formation of oil-water droplets. The surfactants are amphiphilic in nature and are capable of dissolving or solubilizing relatively high amounts of hydrophobic drug compounds.

Non-limiting examples, include, Tween, Dimethylacetamide (DMA), Dimethyl sulfoxide (DMSO), Ethanol, Glycerin, N-methyl-2-pyrrolidone (NMP), PEG 300, PEG 400, Poloxamer 407, Propylene glycol, Phospholipids, Hydrogenated soy phosphatidylcholine (HSPC), Distearoylphosphatidylglycerol (DSPG), L- α -dimyristoylphosphatidylcholine (DMPC), L- α -dimyristoylphosphatidylglycerol (DMPG), Polyoxyl 35 castor oil (CREMOPHOR EL, CREMOPHOR ELP), Polyoxyl 40 hydrogenated castor oil (Cremophor RH 40), Polyoxyl 60 hydrogenated castor oil (CREMOPHOR RH 60), Polysorbate 20 (TWEEN 20), Polysorbate 80 (TWEEN 80), d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS), Solutol HS-15, Sorbitan monooleate (SPAN 20), PEG 300 caprylic/capric glycerides (SOFTIGEN 767), PEG 400 caprylic/capric glycerides (LABRASOL), PEG 300 oleic glycerides (LABRAFIL M-1944CS), Polyoxyl 35 Castor oil (ETOCAS 35), Glyceryl Caprylate (Mono- and Diglycerides) (IMWITOR), PEG 300 linoleic glycerides (LABRAFIL M-2125CS), Polyoxyl 8 stearate (PEG 400 monostearate), Polyoxyl 40 stearate (PEG 1750 monostearate), and combinations thereof. Additionally, suitable surfactants include, for example, polyoxyethylene derivative of sorbitan monolaurate such as polysorbate, caprylcaproyl macrogol glycerides, polyglycolized glycerides, and the like.

According to embodiments, the non-ionic surfactant is selected from one or more of glycerol and polyethylene glycol esters of long chain fatty acids, for example, lauroyl macrogol-32 glycerides or lauroyl polyoxyl-32 glycerides,

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commercially available as GELUCIRE, including, for example, GELUCIRE 39/01 (glycerol esters of saturated C12-C18 fatty acids), GELUCIRE 43/01 (hard fat NF/JPE) and GELUCIRE 50/13 (stearoyl macrogol-32 glycerides EP, 5 stearoyl polyoxyl-32 glycerides NF, stearoyl polyoxylglycerides (USA FDA IIG)). These surfactants may be used at concentrations greater than about 0.01%, and typically in various amounts of about 0.01%-10.0%, 10.1%-20%, and 20.1%-30%. In some embodiments, surfactants may be used at concentrations of about 1% to about 10% (e.g., about 1% to about 5%, about 2% to about 4%, about 3% to about 8%).

According to embodiments, non-ionic surfactants include, for example and without limitation: one or more of oleic acid, linoleic acid, palmitic acid, and stearic acid. According to 15 embodiments, non-ionic surfactants comprise polyethylene sorbitol esters, including polysorbate 80, which is commercially available under the trademark TWEEN® 80 (polysorbate 80) (Sigma Aldrich, St. Louis, Mo.). Polysorbate 80 comprises approximately 60%-70% oleic acid with the remainder comprising primarily linoleic acids, palmitic acids, and stearic acids. Polysorbate 80 may be used in amounts ranging from about 5 to 50%, and according to 20 embodiments, about 30% of the pharmaceutical composition total mass.

According to embodiments, the non-ionic surfactant includes PEG-6 stearate and ethylene glycol palmitostearate, which are available commercially as TEFOSE® 63 (GAT-TEFOSSÉ SAS, Saint-Priest, France), which can be used with, for example, CAPMUL MCM having ratios of MCM to TEFOSE 63 of, for example, 8:2 or 9:1. According to 30 embodiments, other solubilizing agents/non-ionic surfactants combinations include, for example, MIGLYOL 812: GELUCIRE 50/13 or MIGLYOL 812:TEFOSE 63.

According to embodiments, the surfactant can be an anionic surfactant, for example: ammonium lauryl sulfate, dioctyl sodium sulfosuccinate, perfluoro-octane sulfonic acid, potassium lauryl sulfate, or sodium stearate. Cationic surfactants are also contemplated.

According to embodiments, non-ionic or anionic surfactants can be used alone with at least one solubilizing agent or can be used in combination with other surfactants. Accordingly, such surfactants, or any other excipient as set forth herein, may be used to solubilize estradiol. The combination of solubilizing agent, surfactant, and other excipients should be designed whereby the estradiol is absorbed into the vaginal tissue. According to embodiments, the pharmaceutical composition will result in minimal vaginal discharge.

According to embodiments, the pharmaceutical composition further comprises at least one thickening agent. Generally, a thickening agent is added when the viscosity of the pharmaceutical composition results less than desirable absorption. According to embodiments, the surfactant(s) disclosed herein may also provide thickening of the pharmaceutical composition that, upon release, will aid the estradiol in being absorbed by the vaginal mucosa while minimizing vaginal discharge. Examples of thickening agents include: hard fats; propylene glycol; a mixture of hard fat EP/NF/JPE, glyceryl ricinoleate, ethoxylated fatty alcohols (ceteth-20, steareth-20) EP/NF (available as OVUCIRE® 3460, GATTEFOSSÉ, Saint-Priest, France); a mixture of hard fat EP/NF/JPE, glycerol monooleate (type 40) EP/NF (OVUCIRE WL 3264; a mixture of hard fat EP/NF/JPE, glyceryl monooleate (type 40) EP/NF (OVUCIRE WL 2944); a non-ionic surfactant comprising PEG-6 stearate, ethylene glycol palmitostearate, and PEG-32 stearate; TEFOSE 63 or a similar product; and a mixture of various

hard fats (WITEPSOL®, Sasol Germany GmbH, Hamburg, Germany). Other thickening agents such as the alginates, certain gums such as xanthan gums, agar-agar, iota carrageenans, kappa carrageenans, etc. Several other compounds can act as thickening agents like gelatin, and polymers like HPMC, PVC, and CMC. According to embodiments, the viscosity of pharmaceutical compositions in accordance with various embodiments may comprise from about 50 cps to about 1000 cps at 25° C. A person of ordinary skill in the art will readily understand and select from suitable thickening agents.

According to embodiments, the thickening agent is a non-ionic surfactant. For example, polyethylene glycol saturated or unsaturated fatty acid ester or diester is the non-ionic surfactant thickening agent. In embodiments, the non-ionic surfactant comprises a polyethylene glycol long chain (C16-C20) fatty acid ester and further comprises an ethylene glycol long chain fatty acid ester, such as PEG-fatty acid esters or diesters of saturated or unsaturated C16-C18 fatty acids, e.g., oleic, lauric, palmitic, and stearic acids. In embodiments, the non-ionic surfactant comprises a polyethylene glycol long chain saturated fatty acid ester and further comprises an ethylene glycol long chain saturated fatty acid ester, such as PEG- and ethylene glycol-fatty acid esters of saturated C16-C18 fatty acids, e.g., palmitic and stearic acids. Such non-ionic surfactant can comprise PEG-6 stearate, ethylene glycol palmitostearate, and PEG-32 stearate, such as but not limited to TEFOSE 63.

According to embodiments, the non-ionic surfactant used as a thickening agent is not hydrophilic and has good emulsion properties. An illustrative example of such surfactant is TEFOSE 63, which has a hydrophilic-lipophilic balance (HLB) value of about 9-10.

According to embodiments, the pharmaceutical composition further comprises one or more mucoadherent agents to improve vaginal absorption of the estradiol. For example, a mucoadherent agent can be present to aid the pharmaceutical composition with adherence to the mucosa upon activation with water. According to embodiments, polycarbophil is the mucoadherent agent. According to embodiments, other mucoadherent agents include, for example and without limitation: poly (ethylene oxide) polymers having a molecular weight of from about 100,000 to about 900,000; chitosans carbopols including polymers of acrylic acid cross-linked with allyl sucrose or allyl pentaerythritol; polymers of acrylic acid and C10-C30 alkyl acrylate crosslinked with allyl pentaerythritol; carbomer homopolymer or copolymer that contains a block copolymer of polyethylene glycol and a long chain alkyl acid ester; and the like. According to embodiments, various hydrophilic polymers and hydrogels may be used as the mucoadherent agent. According to certain embodiments, the polymers or hydrogels can swell in response to contact with vaginal tissue or secretions, enhancing moisturizing and mucoadherent effects. The selection and amount of hydrophilic polymer may be based on the selection and amount of solubilizing agent. In some embodiments, the pharmaceutical composition includes a hydrophilic polymer but optionally excludes a gelling agent. In embodiments having a hydrogel, from about 5% to about 10% of the total mass may comprise the hydrophilic polymer. In further embodiments, hydrogels may be employed. A hydrogel may comprise chitosan, which swell in response to contact with water. In various embodiments, a cream pharmaceutical composition may comprise PEG-90M. In some embodiments, a mucoadherent agent is present in the pharmaceutical formulation, in the soft gel capsule, or both.

According to embodiments, the pharmaceutical compositions include one or more thermoreversible gels, typically of the hydrophilic nature including for example and without limitation, hydrophilic sucrose and other saccharide-based monomers (U.S. Pat. No. 6,018,033, which is incorporated by reference).

According to embodiments, the pharmaceutical composition further comprises a lubricant. In some embodiments, a lubricant can be present to aid in formulation of a dosage form. For example, a lubricant may be added to ensure that capsules or tablets do not stick to one another during processing or upon storage. Any suitable lubricant may be used. For example, lecithin, which is a mixture of phospholipids, is the lubricant.

According to embodiments, the pharmaceutical composition further comprises an antioxidant. Any suitable antioxidant may be used. For example, butylated hydroxytoluene, butylated hydroxyanisole, and Vitamin E TPGS.

According to embodiments, the pharmaceutical composition comprises about 20% to about 80% solubilizing agent by weight, about 0.1% to about 5% lubricant by weight, and about 0.01% to about 0.1% antioxidant by weight.

The choice of excipient will depend on factors such as, for example, the effect of the excipient on solubility and stability. Additional excipients used in various embodiments may include colorants and preservatives. Examples of colorants include FD&C colors (e.g., blue No. 1 and Red No. 40), D&C colors (e.g., Yellow No. 10), and opacifiers (e.g., Titanium dioxide). According to embodiments, colorants, comprise about 0.1% to about 2% of the pharmaceutical composition by weight. According to embodiments, preservatives in the pharmaceutical composition comprise methyl and propyl paraben, in a ratio of about 10:1, and at a proportion of about 0.005% and 0.05% by weight.

Generally, the solubilizing agents, excipients, other additives used in the pharmaceutical compositions described herein, are non-toxic, pharmaceutically acceptable, compatible with each other, and maintain stability of the pharmaceutical composition and the various components with respect to each other. Additionally, the combination of various components that comprise the pharmaceutical compositions will maintain will result in the desired therapeutic effect when administered to a subject.

Solubility of Estradiol

According to embodiments, solubilizing agents comprising mixtures of medium chain fatty acid glycerides, e.g., C₆-C12, C₈-C12, or C₈-C10 fatty acid mono- and diglycerides or mono-, di-, and triglycerides dissolve estradiol. As illustrated in the Examples, good results were obtained with solubilizing agents that are predominantly a mixture of C8-C10 saturated fatty acid mono- and diglycerides, or medium chain triglycerides (e.g., Miglyol 810 or 812). Longer chain glycerides appear to be not as well suited for dissolution of estradiol.

A solubilizing agent comprising propylene glycol monocaprylate (e.g., CAPRYOL) and 2-(2-Ethoxyethoxy)ethanol (e.g., TRANSCUTOL) solubilized estradiol well.

Manufacture of the Pharmaceutical Composition

According to embodiments, the pharmaceutical composition is prepared via blending estradiol with a pharmaceutically acceptable solubilizing agent, including for example and without limitation, at least one medium chain fatty acid such as medium chain fatty acids consisting of at least one mono-, di-, or triglyceride, or derivatives thereof, or combinations thereof. According to embodiments, the pharma-

ceutical composition also comprises at least one glycol or derivatives thereof or combinations thereof or combinations of at least one glyceride and glycol. The glycol(s) may be used as solubilizing agents or to adjust viscosity and, thus, may be considered thickening agents, as discussed further herein. Optionally added are other excipients including, for example and without limitation, anti-oxidants, lubricants, and the like. According to embodiments, the pharmaceutical composition comprises sufficient solubilizing agent to fully solubilize the estradiol. It is expressly understood, however, the other volumes of solubilizing agent can be used depending on the level of estradiol solubilization desired. Persons of ordinary skill in the art will know and understand how to determine the volume of solubilizing agent and other excipients depending on the desired percent of estradiol to be solubilized in the pharmaceutical composition.

In illustrative embodiments, GELUCIRE 44/14 (lauroyl macrogol-32 glycerides EP, lauroyl polyoxyl-32 glycerides NF, lauroyl polyoxyglycerides (USA FDA IIG)) is heated to about 65° C. and CAPMUL MCM is heated to about 40° C. to facilitate mixing of the oil and non-ionic surfactant, although such heating is not necessary to dissolve the estradiol.

Specific Examples disclosed herein provide additional principles and embodiments illustrating the manufactures of the pharmaceutical compositions disclosed herein.

Delivery Vehicle

Generally, the pharmaceutical compositions described herein delivered intravaginally inside of a delivery vehicle, for example a capsule. According to embodiments, the capsules are soft capsules made of materials well known in the pharmaceutical arts, for example, gelatin. However, according to embodiments, the delivery vehicle is integral with the pharmaceutical composition (i.e., the pharmaceutical composition is the delivery vehicle). In such embodiments the pharmaceutical compositions is a gel, cream, ointment, tablet, or other preparation that is directly applied and absorbed vaginally.

According to embodiments, pharmaceutical compositions disclosed herein are contained in capsules, such as soft gelatin capsules. According to embodiments, the capsules contain one or more of the following: hydrophilic gel-forming bioadhesive (e.g., mucoadhesive) agents; a lipophilic agent; a gelling agent for the lipophilic agent, or a hydrodispersible agent. According to embodiments, the hydrophilic gel-forming bioadhesive agent is carboxyvinyllic acid; hydroxypropylcellulose; carboxymethylcellulose; gelatin; xanthane gum; guar gum; aluminum silicate; or mixtures thereof. According to embodiments, the lipophilic agent is a liquid triglyceride; solid triglyceride (e.g., with a melting point of about 35° C.); carnauba wax; cocoa butter; or mixtures thereof. According to embodiments, the gelling agent is a hydrophobic colloidal silica. According to embodiments, the hydrodispersible agent is: polyoxyethylene glycol; polyoxyethylene glycol 7-glyceryl-cocotate; or mixtures thereof.

According to embodiments, the delivery vehicle is designed for ease of insertion. According to embodiments, the delivery vehicle is sized whereby it can be comfortably inserted into the vagina. According to embodiments, the delivery vehicle is prepared in a variety of geometries. For example, the delivery vehicle is shaped as a tear drop, a cone with frustoconical end, a cylinder, a cylinder with larger “cap” portion, or other shapes suitable for and that ease insertion into the vagina. According to embodiments, deliv-

ery vehicle is used in connection with an applicator. According to other embodiments, delivery vehicle is inserted digitally.

With reference to FIG. 2, delivery vehicle 200 comprises pharmaceutical composition 202 and capsule 204. Width 208 represents the thickness of capsule 204, for example about 0.108 inches. The distance from one end of delivery vehicle 200 to another is represented by distance 206, for example about 0.690 inches. The size of delivery vehicle 200 may also be described by the arc swept by a radius of a given length. For example, arc 210, which is defined by the exterior of gelatin 204, is an arc swept by a radius of about 0.189 inches. Arc 212, which is defined by the interior of capsule 204, is an arc swept by a radius of about 0.0938 inches. Arc 214, which is defined by the exterior of gelatin 204 opposite arc 210, is an arc swept by a radius of about 0.108 inches. Suitable capsules of other dimensions may be provided. According to embodiments, capsule 204 has dimensions the same as or similar to the ratios as provided above relative to each other.

According to embodiments, the delivery vehicle is designed to remaining in the vagina until the pharmaceutical compositions are released. According to embodiments, delivery vehicle dissolves intravaginally and is absorbed into the vaginal tissue with the pharmaceutical composition, which minimizes vaginal discharge. In such embodiments, delivery mechanism is made from constituents that are non-toxic, for example, gelatin.

Design Factors for Vaginally Inserted Pharmaceutical Compositions

According to embodiments, the pharmaceutical composition is designed to maximize favorable characteristics that lead to patient compliance (patients that discontinue treatment prior to completion of the prescribed course of therapy), without sacrificing efficacy. Favorable characteristics include, for example, lack of or reduction of irritation relative to other hormone replacement pessaries, lack of or reduction in vaginal discharge of the pharmaceutical composition and delivery vehicle relative to other hormone replacement pessaries, lack of or reduction of pharmaceutical composition or delivery vehicle residue inside the vagina, ease of administration compared to other hormone replacement pessaries, or improved efficacy of drug product relative to otherwise similar pharmaceutical compositions.

According to embodiments, the pharmaceutical composition is non-irritating or minimizes irritation. Patient irritation comprises pain, pruritis (itching), soreness, excessive discharge, swelling, or other similar conditions. Patient irritation results in poor compliance. Non-irritating or reduced irritation pharmaceutical compositions are measured relative to competing hormone pessaries, including tablets, creams, or other intravaginal estrogen delivery forms.

According to embodiments, the pharmaceutical compositions does not result in systemic exposure (e.g., blood circulation of estradiol), which improves safety. According to other embodiments, the pharmaceutical compositions disclosed herein result in significantly reduced systemic exposure (e.g., blood circulation of estradiol) when compared to RLDs.

According to embodiments, the pharmaceutical composition does not leave residue inside the vagina. Rather, the pharmaceutical composition and delivery vehicle are substantially absorbed or dispersed without resulting in unabsorbed residue or unpleasant sensations of non-absorbed or

non-dispersed drug product. Measurement of lack of residue is relative to other vaginally inserted products or can be measured objectively with inspection of the vaginal tissues. For example, certain other vaginally inserted products contain starch which can result in greater discharge from the vagina following administration than. In some embodiments, the pharmaceutical compositions provided herein provide a lower amount, duration, or frequency of discharge following administration compared to other vaginally inserted products (e.g., compressed tablets).

According to embodiments, the pharmaceutical composition improves vaginal discharge compared to other pessaries, including pessaries that deliver hormones. Ideally, vaginal discharge is eliminated, minimized, or improved compared to competing products.

According to embodiments, the pharmaceutical compositions disclosed herein are inserted digitally. According to embodiments, the pharmaceutical compositions are digitally inserted approximately two inches into the vagina without a need for an applicator. According to embodiments, the pharmaceutical compositions are designed to be also inserted with an applicator, if desired. According to some embodiments, because the site of VVA is in the proximal region of the vagina (towards the vaginal opening), the pharmaceutical compositions disclosed herein are designed to be inserted in the proximal portion of the vagina.

Through extensive experimentation, various medium chain fatty acid esters of glycerol and propylene glycol demonstrated one or more favorable characteristics for development as a human drug product. According to embodiments, the solubilizing agent was selected from at least one of a solvent or co-solvent. Suitable solvents and co-solvents include any mono-, di- or triglyceride and glycols, and combinations thereof.

According to embodiments, the pharmaceutical composition is delivered via a gelatin capsule delivery vehicle. According to these embodiments, the pharmaceutical composition is a liquid pharmaceutical composition. According to embodiments, the delivery vehicle is a soft capsule, for example a soft gelatin capsule. Thus, the pharmaceutical composition of such embodiments is encapsulated in the soft gelatin capsule or other soft capsule.

According to embodiments, the pharmaceutical composition comprises estradiol that is at least about 80% solubilized in a solubilizing agent comprising one or more C6 to C14 medium chain fatty acid mono-, di-, or triglycerides and, optionally, a thickening agent. According to embodiments, the pharmaceutical composition comprises estradiol that is at least about 80% solubilized one or more C6 to C12 medium chain fatty acid mono-, di-, or triglycerides, e.g., one or more C6 to C14 triglycerides, e.g., one or more C6 to C12 triglycerides, such as one or more C8-C10 triglycerides. These embodiments specifically contemplate the estradiol being at least 80% solubilized. These embodiments specifically contemplate the estradiol being at least 90% solubilized. These embodiments specifically contemplate the estradiol being at least 95% solubilized. These embodiments specifically contemplate the estradiol being fully solubilized.

As noted above, liquid pharmaceutical compositions are liquid at room temperature or at body temperature. For example, in some embodiments, a pharmaceutical composition provided herein is a liquid formulation contained within a soft gel capsule. Gels, hard fats, or other solid forms that are not liquid at room or body temperature are less desirable in embodiments of the pharmaceutical composition that are liquid.

The thickening agent serves to increase viscosity, e.g., up to about 10,000 cP (10,000 mPa-s), typically to no more than about 5000 cP, and more typically to between about 50 and 1000 cP. In embodiments, the non-ionic surfactant, e.g., GELUCIRE or TEFOSE, may be solid at room temperature and require melting to effectively mix with the solubilizing agent. However, in these embodiments, the resultant pharmaceutical composition remains liquid, albeit with greater viscosity, not solid.

According to embodiments, the pharmaceutical composition comprises estradiol, the medium chain solubilizing agent, and the thickening agent as the ingredients delivered via a soft capsule delivery vehicle. Other ingredients, e.g., colorants, antioxidants, preservatives, or other ingredients may be included as well.

However, the addition of other ingredients should be in amounts that do not materially change the solubility of the estradiol, the pharmacokinetics of the pharmaceutical composition, or efficacy of the pharmaceutical composition. Other factors that should be considered when adjusting the ingredients of the pharmaceutical composition include the irritation, vaginal discharge, intravaginal residue, and other relevant factors, for example those that would lead to reduced patient compliance. Other contemplated ingredients include: oils or fatty acid esters, lecithin, mucoadherent agents, gelling agents, dispersing agents, or the like.

Methods

According to embodiments, the pharmaceutical compositions disclosed herein can be used for the treatment of VVA, including the treatment of at least one VVA symptom including: vaginal dryness, vaginal or vulvar irritation or itching, dysuria, dyspareunia, and vaginal bleeding associated with sexual activity, among others. According to embodiments the methods of treatment are generally applicable to females.

According to embodiments, the pharmaceutical compositions disclosed herein can be used for the treatment of estrogen-deficient urinary states. According to embodiments, the pharmaceutical compositions disclosed herein can be used for the treatment of dyspareunia, or vaginal bleeding associated with sexual activity.

According to embodiments, treatment of the VVA, estrogen-deficient urinary states, and dyspareunia and vaginal bleeding associated with sexual activity occurs by administering the pharmaceutical compositions intravaginally. According to embodiments where the delivery vehicle is a capsule, the patient obtains the capsule and inserts the capsule into vagina, where the capsule dissolves and the pharmaceutical composition is released into the vagina where it is absorbed into the vaginal tissue. In some embodiments, the pharmaceutical composition is completely absorbed into the vaginal tissue. In some embodiments, the pharmaceutical composition is substantially absorbed into the vaginal tissue (e.g., at least about 80% by weight, at least about 85% by weight, at least about 90% by weight, at least about 95% by weight, at least about 97% by weight, at least about 98% by weight, or at least about 99% by weight of the composition is absorbed). According to embodiments, the capsule is inserted about two inches into the vagina digitally, however the depth of insertion is generally any depth that allows for adsorption of substantially all of the pharmaceutical composition. According to embodiments, the capsule can also be applied using an applicator that deposits the capsule at an appropriate vaginal depth as disclosed herein.

According to embodiments where the pharmaceutical composition is a cream, gel, ointment, or other similar preparation, the pharmaceutical composition is applied digitally, as is well known and understood in the art.

Upon release of the pharmaceutical composition in the vagina, estradiol is locally absorbed. For example, following administration of the pessary to the proximal region of the vagina of a patient provides a therapeutically effective concentration of estradiol over 24 hours in the proximal region of the vagina.

According to embodiments, the timing of administration of the pharmaceutical composition of this disclosure may be conducted by any safe means as prescribed by an attending physician. According to embodiments, a patient will administer the pharmaceutical composition (e.g., a capsule) intravaginally each day for 14 days, then twice weekly thereafter.

According to embodiments, the pharmaceutical compositions are vaginally administered with co-administration of an orally administered estrogen-based (or progestin-based or progestin- and estrogen-based) pharmaceutical drug product, or patch, cream, gel, spray, transdermal delivery system or other parenterally-administered estrogen-based pharmaceutical drug product, each of which can include natural, bio-similar, or synthetic or other derived estrogens or progestins. According to embodiments, modulation of circulating estrogen levels provided via the administration of the pharmaceutical compositions disclosed herein, if any, are not intended to be additive to any co-administered estrogen product and its associated circulating blood levels. According to other embodiments, co-administered estrogen products are intended to have an additive effect as would be determined by the patient physician.

According to embodiments, the efficacy and safety of the pharmaceutical compositions described herein in the treatment of the symptoms of VVA may be determined. According to embodiments, the size, effect, cytology, histology, and variability of the VVA may be determined using various endpoints to determine efficacy and safety of the pharmaceutical compositions described herein or as otherwise accepted in the art, at present or as further developed. On source of endpoints is with the US Food and Drug Administration's (FDA) published guidelines for treatment of VVA with estradiol.

Measurement of Efficacy

According to embodiments, administration of the pharmaceutical compositions described herein resulted in treatment of the VVA, as well as improvement of one or more of the associated symptoms. Patients with VVA experience shrinking of the vaginal canal in both length and diameter and the vaginal canal has fewer glycogen-rich vaginal cells to maintain moisture and suppleness. In addition, the vaginal wall can become thin, pale, dry, or sometimes inflamed (atrophic vaginitis). These changes can manifest as a variety of symptoms collectively referred to as VVA. Such symptoms include, without limitations, an increase in vaginal pH; reduction of vaginal epithelial integrity, vaginal secretions, or epithelial surface thickness; pruritis; vaginal dryness; dyspareunia (pain or bleeding during sexual intercourse); urinary tract infections; or a change in vaginal color. According to embodiments, efficacy is measured as a reduction of vulvar and vaginal atrophy in a patient back to premenopausal conditions. According to embodiments, the change is measured as a reduction in the severity of one or more atrophic effects measured at baseline (screening, Day 1) and compared to a measurement taken at Day 15 (end of

treatment). Severity of the atrophic effect may be measured using a scale of 0 to 3 where, for example, none=0, mild=1, moderate=2, or severe=3. Such scoring is implemented to evaluate the pre-treatment condition of patients; to determine the appropriate course of a treatment regime; such as dosage, dosing frequency, and duration, among others; and post-treatment outcomes.

One of the symptoms of VVA is increased vaginal pH. In further aspects of this disclosure, treatment with the pharmaceutical compositions described herein resulted in a decrease in vaginal pH. A decrease in vaginal pH is measured as a decrease from the vaginal pH at baseline (screening) to the vaginal pH at Day 15, according to embodiments. In some embodiments, a pH of 5 or greater may be associated with VVA. In some embodiments, pH is measured using a pH indicator strip placed against the vaginal wall. In some embodiments, a change in vaginal pH is a change in a patient's vaginal pH to a pH of less than about pH 5.0. In some embodiments, a subject's vaginal pH may be less than about pH 4.9, pH 4.8, pH 4.7, pH 4.6, pH 4.5, pH 4.4, pH 4.3, pH 4.2, pH 4.1, pH 4.0, pH 3.9, pH 3.8, pH 3.7, pH 3.6, or pH 3.5.

According to embodiments, treatment with the pharmaceutical compositions described herein resulted in improvements in the vaginal Maturation Index. The Maturation Index is measured as a change in cell composition. According to embodiments and as related to VVA, a change in cell composition is measured as the change in percent of composition or amount of parabasal vaginal cells, intermediate cells, and superficial vaginal cells, such as a change in the composition or amount of parabasal vaginal cells compared with or, relative to, a change in superficial vaginal cells. A subject having VVA symptoms often has an increased number of parabasal cells and a reduced number of superficial cells (e.g., less than about 5%) compared with women who do not suffer from VVA. Conversely, a subject having decreasing VVA symptoms, or as otherwise responding to treatment, may demonstrate an improvement in the Maturation Index, specifically a decrease in the amount of parabasal cells or an increase in the amount of superficial cells compared to baseline (screening). In embodiments, a decrease in parabasal cells is measured as a reduction in the percent of parabasal cells; the percent reduction may be at least about an 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15% or 10% reduction in the number of parabasal cells. In embodiments, a percent reduction may be at least about a 54% reduction in the number of parabasal cells. In embodiments, an increase in superficial cells is measured as an increase in the percent of superficial cells; the percent increase in superficial cells may be at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% increase in the number of superficial cells. In further embodiments, a percent increase may be at least about a 35% increase in the number of superficial cells.

In some embodiments, an improvement in the Maturation Index is assessed as a change over time. For example, as a change in cell composition measured at a baseline (screening) at Day 1 compared to the cell composition measured at Day 15. The change in cell composition may also be assessed as a change in the amount of parabasal cells over time, optionally in addition to measuring changes in parabasal cells and superficial cells as described above. Such cells may be obtained from the vaginal mucosal epithelium through routine gynecological examination and examined by means of a vaginal smear.

In various further aspects of this disclosure, treatment with the pharmaceutical compositions described herein

resulted in any of: an increase in superficial cells; a decrease in parabasal cells; and an increase in intermediate cells.

In further aspects of this disclosure, samples may be collected to determine hormone levels, in particular, estradiol levels. In some embodiments, blood samples may be taken from a subject and the level of estradiol measured (pg/ml). In some embodiments, estradiol levels may be measured at 0 hours (for example, at time of first treatment), at 1 hour (for example, post first treatment), at 3 hours, and at 6 hours. In some embodiments, samples may be taken at day 8 (for example, post first treatment) and at day 15 (for example, one day post the last treatment on day 14). In some embodiments, descriptive statistics of plasma estradiol concentrations at each sampling time and observed C_{max} and T_{max} values may be measured and the AUC calculated.

In some embodiments, a pessary can comprise about 25 μ g of estradiol. In such cases, administration of the pessary to a patient can provide, in a plasma sample from the patient, parameters including one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 19 pg/ml to about 29 pg/ml (e.g., 19.55 pg/ml to about 28.75 pg/ml); or 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 75 pg*hr/ml to about 112 pg*hr/ml (e.g., 75.82 pg*hr/ml to about 111.50). In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 9 pg/ml to about 14 pg/ml (e.g., 9.17 pg/ml to about 13.49 pg/ml); and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 43 pg*hr/ml to about 65 pg*hr/ml (e.g., 43.56 pg*hr/ml to about 64.06 pg*hr/ml). In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, provides one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 416 pg/ml to about 613 pg/ml (e.g., 416.53 pg/ml to about 612.55 pg/ml); and 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 3598 pg*hr/ml to about 5291 pg*hr/ml (e.g., 3598.04 pg*hr/ml to about 5291.24 pg*hr/ml).

In some embodiments, a pessary can comprise about 10 μ g of estradiol. In such cases, administration of the pessary to a patient can provide, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 12 pg/ml to about 18 pg/ml (e.g., 12.22 pg/ml to about 17.98 pg/ml); 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 42 pg*hr/ml to about 63 pg*hr/ml (e.g., 42.18 pg*hr/ml to about 62.02 pg*hr/ml); and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estradiol of about 1 hrs to about 3 hrs (e.g., 1.49 hrs to about 2.19 hrs). In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 4 pg/ml to about 7 pg/ml (e.g., 4.38 pg/ml to about 6.44 pg/ml); 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 20 pg*hr/ml to about 31 pg*hr/ml (e.g., 20.60 pg*hr/ml to about 30.30 pg*hr/ml); and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone of about 4 hrs to about 8 hrs (e.g., 4.99 hrs to about 7.34 hrs). In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, one or more parameters

selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 10 pg/ml to about 16 pg/ml (e.g., 10.34 pg/ml to about 15.20 pg/ml); 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 56 pg*hr/ml to about 84 pg*hr/ml (e.g., 56.61 pg*hr/ml to about 83.25 pg*hr/ml); and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone sulfate of about 4 hrs to about 7 hrs (e.g., 4.67 hrs to about 6.86 hrs).

In some embodiments, a pessary can comprise about 4 μ g of estradiol. In such cases, administration of the pessary to a patient can provide, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estradiol of about 4 pg/ml to about 8 pg/ml; 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol of about 16 pg*hr/ml to about 26 pg*hr/ml; and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estradiol of about 0.25 hrs to about 2 hrs. In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone of about 1 pg/ml to about 3 pg/ml; 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone of about 8 pg*hr/ml to about 13 pg*hr/ml; and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone of about 1 hrs to about 4 hrs. In some embodiments, administration of the pessary to a patient provides, in a plasma sample from the patient, one or more parameters selected from: 1) a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate of about 4 pg/ml to about 7 pg/ml; 2) a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate of about 22 pg*hr/ml to about 34 pg*hr/ml; and 3) a corrected geometric mean time to peak plasma concentration (T_{max}) of estrone sulfate of about 1 hrs to about 3 hrs.

A pharmaceutical composition provided herein can result in substantially local delivery of estradiol. For example, plasma concentrations of estradiol, estrone, and estrone sulfate measured in the plasma of a patient following administration of a pharmaceutical composition as provided herein be statistically similar to those measured following administration of a placebo formulation (i.e. a similar formulation lacking the estradiol). Accordingly, in some embodiments, the plasma concentrations of estradiol, estrone, or estrone sulfate measured following administration of a pharmaceutical composition provided herein may be low compared to RLD formulations.

In some embodiments, a pessary can include about 1 μ g to about 25 μ g of estradiol. Upon administration of the pessary to a patient, a plasma sample from the patient can provide a corrected geometric mean peak plasma concentration (C_{max}) of estradiol that is less than about 30 pg/ml. For example, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estradiol that is less than about 18 pg/ml. In some embodiments, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol that is less than about 112 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estradiol that is less than about 63 pg*hr/ml.

In some embodiments, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone that is less than about 14 pg/ml. For example, administration of the pessary to a

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patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone that is less than about 7 pg/ml. In some embodiments, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone that is less than about 65 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone that is less than about 31 pg*hr/ml.

In some embodiments, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate that is less than about 613 pg/ml. For example, administration of the pessary to a patient provides a corrected geometric mean peak plasma concentration (C_{max}) of estrone sulfate that is less than about 16 pg/ml. In some embodiments, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate that is less than about 5291 pg*hr/ml. For example, administration of the pessary to a patient provides a corrected geometric mean area under the curve (AUC)₀₋₂₄ of estrone sulfate that is less than about 84 pg*hr/ml.

In further aspects of this disclosure, capsule disintegration may be determined. In some embodiments, delivery vehicle disintegration or absorption (presence or absence of the delivery vehicle after administration) at day 1 of treatment (for example, at 6 hours post first treatment) and at day 15 (for example, one day post the last treatment on day 14).

Statistical Measurements

According to embodiments, pharmacokinetics of the pharmaceutical composition disclosed herein are measured using statistical analysis. According to embodiments, Analysis of Variance ("ANOVA") or Analysis of CoVariance ("ANCOVA") are used to evaluate differences between a patient receiving treatment with a pharmaceutical composition comprising an active pharmaceutical composition (for example, a pharmaceutical composition comprising estro-

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diol) and a patient receiving treatment with a placebo (for example, the same pharmaceutical composition but without estradiol) or a reference drug. A person of ordinary skill in the art will understand how to perform statistical analysis of the data collected.

EXAMPLES

The following examples are of pharmaceutical compositions, delivery vehicles, and combinations thereof. Methods of making are also disclosed. Data generated using the pharmaceutical compositions disclosed herein are also disclosed.

Example 1: Pharmaceutical Composition

In embodiments, estradiol is procured and combined with one or more pharmaceutically acceptable solubilizing agents. The estradiol is purchased as a pharmaceutical grade ingredient, often as micronized estradiol, although other forms can also be used. In embodiments, the pharmaceutical composition comprises estradiol in a dosage strength of from about 1 µg to about 50 µg. In embodiments, the pharmaceutical composition comprises 10 µg of estradiol. In embodiments, the pharmaceutical composition comprises 25 µg of estradiol.

In embodiments, the estradiol is combined with pharmaceutically acceptable solubilizing agents, and, optionally, other excipients, to form a pharmaceutical composition. In embodiments, the solubilizing agent is one or more of CAPMUL MCM, MIGLYOL 812, GELUCIRE 39/01, GELUCIRE 43/01, GELUCIRE 50/13, and TEFOSE 63.

GELUCIRE 39/01 and GELUCIRE 43/01 each have an HLB value of 1. GELUCIRE 50/13 has an HLB value of 13. TEFOSE 63 has an HLB value of between 9 and 10.

Various combinations of pharmaceutically acceptable solubilizing agents were combined with estradiol and examined as shown in Table 1.

TABLE 1

Capmul MCM ("MCM"), Gelucire 39/01 ("39/01"), Gelucire 43/01 ("43/01"), Gelucire 50/13 ("50/13"), and Tefose ("Tefose 63")							
#	Vehicle system	Ratio	Physical state @ Room Temperature	Physical state @ 37° C. after ~30 minutes	Viscosity (cps)	Melting Time @ 37° C.	Dispersion in water 37° C.
1	MCM: 39/01	8:2	Solid	Clear liquid	50 @ 37° C.	Start: 6 min Finish: 12 min	Small oil drops on top
2	MCM: 39/01	7:3	Solid	Clear liquid		Start: 9 min Finish: 19 min	
3	MCM: 39/01	6:4	Solid	Clear liquid		Start: 20 min Finish: 32 min	
4	MCM: 43/01	8:2	Solid	Liquid with solid particles			
5	MCM: 43/01	7:3	Solid	Liquid with solid particles			
6	MCM: 50/13	9:1	Liquid/cloudy	Liquid/cloudy	140 @ 25° C.	Clear after 20 min	Uniformly cloudy dispersion
7	MCM: 50/13	8:2	Liquid/cloudy	Liquid/cloudy	190 @ 25° C.		Uniformly cloudy dispersion
8	MCM: 50/13	7:3	Semisolid	Semisolid			
9	MCM: TEFOSE 63	9:1	Semisolid	Liquid/cloudy	150 @ 25° C.	Start: 1 min Finish: 5 min	Uniformly cloudy dispersion

TABLE 1-continued

Capmul MCM ("MCM"), Gelucire 39/01 ("39/01"), Gelucire 43/01 ("43/01"), Gelucire 50/13 ("50/13"), and Tefose ("Tefose 63")						
#	Vehicle system	Ratio	Physical state @ Room Temperature	Physical state @ 37° C. after ~30 minutes	Viscosity (cps)	Melting Time @ 37° C. Dispersion in water 37° C.
10	MCM: TEFOSE 63	8:2	Semisolid	Semisolid	240 @ 25° C.	Uniformly cloudy dispersion
11	MCM: TEFOSE 63	7:3	Semisolid	Semisolid	380 @ 25° C.	Semisolid after 30 min at 37° C., doesn't melt at 41° C. Uniformly cloudy dispersion
12	MIGLYOL 812: 50/13	9:1	Semisolid	Semisolid	140 @ 25° C.	2 phases, oil on top
13	MIGLYOL 812: TEFOSE 63	9:1	Liquid/cloudy	Liquid/cloudy	90 @ 25° C.	Start: 1 min Finish: 5 min 2 phases, oil on top

Pharmaceutical compositions in Table 1 that were liquid or semisolid at room temperature were tested using a Brookfield viscometer (Brookfield Engineering Laboratories, Middleboro, Mass.) at room temperature. Pharmaceutical compositions appearing in Table 1 that were solid at ambient temperature were tested using a Brookfield viscometer at 37° C.

Pharmaceutical compositions appearing in Table 1 that were solid at room temperature were assessed at 37° C. to determine their melting characteristics. The viscosity of the gels can be important during encapsulation of the formulation. For example, in some cases, it is necessary to warm the formulation prior to filling of the gelatin capsules. In addition, the melting characteristics of the composition can have important implications following administration of the formulation into the body. For example, in some embodiments, the formulation will melt at temperatures below about 37° C. Pharmaceutical Composition 11 (Capmul MCM/Tefose 63), for example, did not melt at 37° C. or 41° C.

A dispersion assessment of the pharmaceutical compositions appearing in Table 1 was performed. The dispersion assessment was performed by transferring 300 mg of each vehicle system in 100 ml of 37° C. water, without agitation, and observing for mixing characteristics. Results varied from formation of oil drops on the top to separation of

phases to uniform, but cloudy dispersions. Generally speaking, it is believed that formulations able to readily disperse in aqueous solution will have better dispersion characteristics upon administration. It was surprisingly found, however, as shown below in Examples 7-9, that formulations that did not readily disperse in aqueous solution (e.g., Formulation 13) and instead formed two phases upon introduction to the aqueous solution were found to be the most effective when administered to the human body.

Example 2: Delivery Vehicle

In embodiments, the pharmaceutical composition is delivered in a gelatin capsule delivery vehicle. The gelatin capsule delivery vehicle comprises, for example, gelatin (e.g., Gelatin, NF (150 Bloom, Type B)), hydrolyzed collagen (e.g., GELITA®, GELITA AG, Eberbach, Germany), glycerin, sorbitol special, or other excipients in proportions that are well known and understood by persons of ordinary skill in the art. Sorbitol special may be obtained commercially and may tend to act as a plasticizer and humectant.

A variety of delivery vehicles were developed, as shown in Table 2, Gels A through F. In Table 2, each delivery vehicle A through F differs in the proportion of one or more components.

TABLE 2

Gelatin Capsule Delivery Vehicles						
Ingredient	A % w/w	B % w/w	C % w/w	D % w/w	E % w/w	F % w/w
Gelatin, NF (150 Bloom, Type B)	41.0	41.0	41.0	41.0	43.0	43.0
Glycerin 99.7%, USP	6.0	6.0	6.0	6.0	18.0	18.0
Sorbitol Special, USP	15.0	15.0	15.0	15.0		
GELITA® (hydrolyzed collagen)	3				3.0	
Citric acid		0.1	0.5	1		0.1
Purified Water	35.0	37.9	37.5	37.0	36.0	38.9
Total	100.0	100.0	100.0	100.0	100.0	100.0
Dissolution gel strips, Avg of 3 (500 ml DH2O, 50 rpm @ 37° C.)	48 min (42, 45, 58)	50 min (50, 51, 50)	75 min (76, 75, 74)	70 min (70, 71, 70)		
Dissolution gel strips, Avg of 3 (500 ml pH 4 buffer, 50 rpm @ 37° C.)	70 min				78 min	82 min

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Each delivery vehicle A through F was prepared at a temperature range from about 45° C. to about 85° C. Each molten delivery vehicle A through F was cast into a film, dried, and cut into strips. The strips were cut into uniform pieces weighing about 0.5 g, with about 0.5 mm thickness. Strips were placed into a USP Type 2 dissolution vessel in either water or pH 4 buffer solution and the time for them to completely dissolve was recorded (see TABLE 2). Delivery vehicle A had the fastest dissolution in both water and pH 4 buffer solution.

Example 3: Pharmaceutical Compositions and Delivery Vehicle

Various combinations of the pharmaceutical compositions from TABLE 1 and from TABLE 2 were prepared. The combinations are shown in TABLE 3.

TABLE 3

Trial	Pharmaceutical Composition	Ratio	Batch Size g	Delivery Vehicle
1	MCM:39/01	8:2	750	A
2	MCM:50/13	8:2	750	A
3	MCM:TEFOSE 63	8:2	750	A
4	MCM:TEFOSE 63	8:2	750	B
5	MIGLYOL 812:TEFOSE 63	9:1	750	A

Each aliquot of the pharmaceutical compositions of Table 3 about 300 mg to about 310 mg. Batch size was as listed in TABLE 3. To encapsulate the vehicle system, each 300 mg to about 310 mg pharmaceutical composition aliquot was encapsulated in about 200 mg of the gelatin capsule delivery vehicle. Thus, for example, in Trial 1, the pharmaceutical composition denoted by MCM:39/01 was encapsulated in gelatin capsule delivery vehicle A for a total encapsulated weight of about 500 mg to about 510 mg. The aliquot size is arbitrary depending on the concentration of the estradiol and the desired gelatin capsule delivery vehicle size. Artisans will readily understand how to adjust the amount of estradiol in the pharmaceutical composition to accommodate a given size of delivery vehicle, when the delivery vehicle encapsulates the pharmaceutical composition.

Example 4: Estradiol Solubility

In various experiments, solubilizing agents were tested to determine whether they were able to solubilize 2 mg of estradiol for a total pharmaceutical composition weight of 100 mg. The solubilizing agents were considered suitable if estradiol solubility in the solubilizing agent was greater than or equal to about 20 mg/g. Initial solubility was measured by dissolving micronized estradiol into various solubilizing agents until the estradiol was saturated (the estradiol/solubilizing agent equilibrated for three days), filtering the undissolved estradiol, and analyzing the resulting pharmaceutical composition for estradiol concentration by HPLC.

TABLE 4

Solubility of Solubilizing Agents (* denotes literature reference)	
Ingredient	Solubility (mg/g)
PEG 400	105*
Propylene Glycol	75*
Polysorbate 80	36*

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TABLE 4-continued

Solubility of Solubilizing Agents (* denotes literature reference)	
Ingredient	Solubility (mg/g)
TRANSCUTOL HP	141
CAPMUL PG8	31.2

Example 5: Pharmaceutical Compositions

The following pharmaceutical compositions are contemplated.

Gel Mass

Ingredient	% w/w	Qty/Batch (kg)
Gelatin 150 Bloom Lined Bone, NF	41.00	82.00
Hydrolyzed Gelatin	3.00	6.00
Glycerin 99.7%	6.00	12.00
Sorbitol Special, NF	15.00	30.00
Opatint White G-18006	1.20	2.40
Opatine Red DG-15001	0.06	0.12
Purified Water, USP	33.74	67.48
Total	100.00	200.00 Kg

Pharmaceutical Composition 1: 10 µg Estradiol

Ingredients	Qty/Capsule (mg)	% w/w	Qty/Batch
Estradiol hemihydrate micronized, USP	0.010	0.003	0.10 g
CAPMUL ® MCM, NF (Glyceryl Caprylate/Caprates or Medium Chain Mono- and Diglycerides)	240.0	79.997	2.40 kg
GELUCIRE ® 50/13 (stearoyl polyoxyl-32 glycerides NF)	60.0	20.0	600.0 g
Total	300.0	100.0	3.0 kg

Pharmaceutical Composition 2: 10 µg Estradiol

Ingredients	Qty/Capsule (mg)	% w/w	Qty/Batch
Estradiol hemihydrate micronized, USP	0.010	0.003	0.10 g
MIGLOYL ® 812 (medium chain triglyceride)	270.0	89.997	2.70 kg
TEFOSE ® 63 (mixture of PEG-6 stearate or ethylene glycol palmitostearate or PEG-32 stearate; polyoxyl 6 and polyoxyl 32 palmitostearate/glycol stearate)	30.0	10.0	300.0 g
Total	300.0	100.0	3.00 kg

Pharmaceutical Composition 3: 25 µg Estradiol

Ingredients	Qty/Capsule (mg)	% w/w	Qty/Batch
Estradiol hemihydrate micronized, USP	0.026*	0.009	0.26 g
MIGLOYL ® 812 (medium chain triglyceride)	270.0	89.991	2.70 kg
TEFOSE ® 63 (mixture of PEG-6 stearate or ethylene glycol	30.02	10.0	300.0 g

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-continued

Ingredients	Qty/Capsule (mg)	% w/w	Qty/Batch
palmitostearate or PEG-32 stearate; polyoxyl 6 and polyoxyl 32 palmitostearate/glycol stearate)			
Total	300.0	100.0	3.00 kg

*1.0 mg estradiol is equivalent to 1.03 mg estradiol hemihydrate

Pharmaceutical Composition 4: 4 µg Estradiol

Ingredients	Qty/Capsule (mg)	% w/w	Qty/Batch
Estradiol hemihydrate micronized, USP	0.0041*	0.001	0.041 g
MIGLOYL ® 812 (medium chain triglyceride)	269.99	89.999	2700.0 g
TEFOSE ® 63 (mixture of PEG-6 stearate or ethylene glycol palmitostearate or PEG-32 stearate; polyoxyl 6 and polyoxyl 32 palmitostearate/glycol stearate)	30.0	10.0	300.0 g
Total	300.0	100.0	3000.0 g

*1.0 mg estradiol is equivalent to 1.03 mg estradiol hemihydrate

Example 6: Process

FIG. 1 illustrates an embodiment of a method making pharmaceutical composition comprising estradiol solubilized in CapmulMCM/Gelucire solubilizing agent encapsulated in a soft gelatin delivery vehicle 100. In operation 102, the CapmulMCM is heated to 40° C.±5° C. Heating may be accomplished through any suitable means. The heating may be performed in any suitable vessel, such as a stainless steel vessel. Other pharmaceutical compositions can be made using the same general method by substituting various excipients, including the solubilizing agent.

In operation 104, GELUCIRE is mixed with the CapmulMCM to form the finished solubilizing agent. As used herein, any form of GELUCIRE may be used in operation 104. For example, one or more of GELUCIRE 39/01, GELUCIRE 43/01, GELUCIRE 50/13 may be used in operation 104. Mixing is performed as would be known to persons of ordinary skill in the art, for example by impeller, agitator, stirrer, or other like devices used to mix pharmaceutical compositions. Operation 104 may be performed under an inert or relatively inert gas atmosphere, such as nitrogen gas. Mixing may be performed in any vessels that are known to persons of ordinary skill in the art, such as a stainless steel vessel or a steel tank.

In operation 106 estradiol is mixed into the solubilizing agent. In embodiments, the estradiol is micronized when mixed into the solubilizing agent. In other embodiments, the estradiol added is in a non-micronized form. Mixing may be facilitated by an impeller, agitator, stirrer, or other like devices used to mix pharmaceutical compositions. Operation 106 may be performed under an inert or relatively inert gas atmosphere, such as nitrogen gas.

In embodiments, however, the addition of estradiol may be performed prior to operation 104. In that regard, operations 104 and 106 are interchangeable with respect to timing or can be performed contemporaneously with each other.

In operation 110, the gelatin delivery vehicle is prepared. Any of the gelatin delivery vehicles described herein may be

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used in operation 110. In embodiments, gelatin, hydrolyzed collagen, glycerin, and other excipients are combined at a temperature range from about 45° C. to about 85° C. and prepared as a film. Mixing may occur in a steel tank or other container used for preparing gelatin delivery vehicles. Mixing may be facilitated by an impeller, agitator, stirrer, or other devices used to combine the contents of gelatin delivery vehicles. Operation 110 may be performed under an inert or relatively inert gas atmosphere, such as nitrogen gas. In embodiments, the gelatin delivery vehicle mixture is degassed prior to being used to encapsulate the pharmaceutical composition.

In operation 112, the gelatin delivery vehicle encapsulates the pharmaceutical composition, according to protocols well known to persons of ordinary skill in the art. In operation 112, a soft gelatin capsule delivery vehicle is prepared by combining the pharmaceutical composition made in operation 106 with the gelatin delivery vehicle made in operation 110. The gelatin may be wrapped around the material, partially or fully encapsulating it or the gelatin can also be injected or otherwise filled with the pharmaceutical composition made in operation 106.

In embodiments, operation 112 is completed in a suitable die to provide a desired shape. Vaginal soft gel capsules may be prepared in a variety of geometries. For example, vaginal soft gel capsules may be shaped as a tear drop, a cone with frustoconical end, a cylinder, a cylinder with larger “cap” portion as illustrated in FIG. 2, or other shapes suitable for insertion into the vagina. The resulting pharmaceutical composition encapsulated in the soft gelatin delivery vehicle may be inserted digitally or with an applicator.

Example 7: Study of Estradiol Pharmaceutical Composition on the Improvement of Vulvovaginal Atrophy (VVA)

The objective of this study was designed to evaluate the efficacy and safety of a pharmaceutical composition comprising 10 µg estradiol (i.e., Pharmaceutical Composition 2) in treating moderate to severe symptoms of VVA associated with menopause after 14 days of treatment, and to estimate the effect size and variability of vulvovaginal atrophy endpoints. In addition, the systemic exposure to estradiol from single and multiple doses of the pharmaceutical composition was investigated.

This study was a phase 1, randomized, double-blind, placebo-controlled trial to evaluate safety and efficacy of the pharmaceutical composition in reducing moderate to severe symptoms of vaginal atrophy associated with menopause and to investigate the systemic exposure to estradiol following once daily intravaginal administrations of a pharmaceutical composition for 14 days.

Postmenopausal subjects who met the study entry criteria were randomized to one of two treatment groups (pharmaceutical composition or placebo). During the screening period subjects were asked to self-assess the symptoms of VVA, including vaginal dryness, vaginal or vulvar irritation or itching, dysuria, vaginal pain associated with sexual activity, and vaginal bleeding associated with sexual activity. Subjects with at least one self-assessed moderate to severe symptom of VVA identified by the subject as being most bothersome to her were eligible to participate in the study.

Clinical evaluations were performed at the following time points:

- Screening Period (up to 28 days);
- Visit 1—Randomization/Baseline (day 1);
- Visit 2—Interim (day 8); and
- Visit 3—End of the treatment (day 15).

Eligible subjects were randomized in a 1:1 ratio to receive either pharmaceutical composition comprising estradiol 10 µg or a matching placebo vaginal softgel capsule, and self-administered their first dose of study medication at the clinical facility under the supervision of the study personnel. Serial blood samples for monitoring of estradiol level were collected at 0.0, 1.0, 3.0, and 6.0 hours relative to first dose administration on day 1. Subjects remained at the clinical site until completion of the 6-hour blood draw and returned to clinical facility for additional single blood draws for measurement of estradiol concentration on day 8 (before the morning dose) and day 15. Subjects were provided with enough study medication until the next scheduled visit and were instructed to self-administer their assigned study treatment once a day intravaginally at approximately the same time (±1 hour) every morning. Each subject was provided with a diary in which she was required to daily record investigational drug dosing dates and times. Subjects returned to clinical facility on day 8 for interim visit and on day 15 for end of treatment assessments and post study examinations. Capsule disintegration state was assessed by the investigator at day 1 (6 hours post-dose) and day 15.

The study involved a screening period of up to 28 days before randomization and treatment period of 14 days. Selection of dosage strength (estradiol 10 µg) and treatment regimen (once daily for two weeks) was based on the FDA findings on safety and efficacy of the RLD.

Number of Subjects (Planned and Analyzed)

Up to 50 (25 per treatment group) postmenopausal female subjects 40 to 75 years old with symptoms of moderate to severe VVA were randomized. 50 subjects were enrolled, 48 subjects completed the study, and 48 subjects were analyzed.

Diagnosis and Main Criteria for Inclusion

Fifty female subjects were enrolled in the study. Postmenopausal female subjects 40 to 75 years of age, with a mean age was 62.3 years were enrolled. Subjects' mean weight (kg) was 71.2 kg with a range of 44.5-100 kg. Subjects' mean height (cm) was 162.6 cm with a range of 149.9-175.2 cm, and the mean BMI (kg/m²) was 26.8 kg/m² with a range of 19-33 kg/m². Criteria of inclusion in the study included: self-identification of at least one moderate to severe symptom of VVA, for example, vaginal dryness, dyspareunia, vaginal or vulvar irritation, burning, or itching, dysuria, vaginal bleeding associated with sexual activity, that was identified by the subject as being most bothersome to her; ≤5% superficial cells on vaginal smear cytology; vaginal pH>5.0; and estradiol level ≤50 pg/ml. Subject who were judged as being in otherwise generally good health on the basis of a pre-study physical examination, clinical laboratory tests, pelvic examination, and mammography were enrolled.

Estradiol 10 µg or Placebo, Dose, and Mode of Administration

Subjects were randomly assigned (in 1:1 allocation) to self-administer one of the following treatments intravaginally once daily for 14 days:

Treatment A: The pharmaceutical composition of Example 5 (Pharmaceutical Composition 2: 10 µg estradiol); or

Treatment B: Placebo vaginal softgel capsule, containing the same formulation as Treatment A, except for the 10 µg of estradiol.

The estradiol formulation was a tear drop shaped light pink soft gel capsule. Treatment B had the same composition, appearance, and route of administration as the Treatment A, but contained no estradiol.

Duration of Treatment

The study involved a screening period of up to 28 days before randomization and a treatment period of 14 days.

Criteria for Evaluation

Efficacy Endpoints:

Change from baseline (screening) to day 15 in the Maturation Index (percent of parabasal vaginal cells, superficial vaginal cells, and intermediate vaginal cells) of the vaginal smear. Data for this endpoint are shown in Tables 6-8.

Change from baseline (screening) to day 15 in vaginal pH. Data for this endpoint are shown in Table 9.

Change from baseline (randomization) to day 15 in severity of the most bothersome symptoms: (1) vaginal dryness; (2) vaginal or vulvar irritation, burning, or itching; (3) dysuria; (4) dyspareunia; (5) vaginal bleeding associated with sexual activity. Data for this endpoint are shown in Tables 13 and 15.

Change from baseline (randomization) to day 15 in investigator's assessment of the vaginal mucosa. Data for this endpoint are shown in Tables 18-21.

Unless otherwise noted, the efficacy endpoints were measured as a change-from Visit 1—Randomization/Baseline (day 1) to Visit 3—End of the treatment (day 15), except for vaginal bleeding which was expressed as either treatment success or failure.

Other endpoints include:

Vital signs, weight, changes in physical exam, pelvic and breast exam, and adverse events were evaluated as part of the safety endpoints.

Concentration of estradiol at each sampling time.

Peak concentration of estradiol on day 1 and sampling time at which peak occurred.

Delivery vehicle disintegration to measure the amount of residual delivery vehicle remains in the vagina post treatment.

Results from the assessment of plasma concentrations of estradiol are presented in Table 5.

TABLE 5

Safety Results: The descriptive statistics for Day 1 plasma estradiol C_{max} and T_{max} are provided below.

	Estradiol 10 µg		Placebo	
	C_{max}	T_{max}	C_{max}	T_{max}
N	24	24	26	26
Mean ± SD	30.7 ± 7.47	2.12 ± 1.73	27.5 ± 17.26	4.00 ± 2.68
Geometric Mean	29.9	—	24.7	—
Median	29.8	1.00	22.1	6.00
Min, Max	19.7, 52.3	1.00, 6.00	15.1, 90.0	0.00, 6.00
CV %	24.3%	81.3%	62.9%	67.1%

Other Endpoints:

Maturation Index Results

Vaginal cytology data was collected as vaginal smears from the lateral vaginal walls according to standard procedures to evaluate vaginal cytology at screening and Visit 3—End of treatment (day 15). The change in the Maturation Index was assessed as a change in cell composition measured at Visit 1—Baseline (day 1) compared to the cell composition measured at Visit 3—End of treatment (day 15). The change in percentage of superficial, parabasal, and intermediate cells obtained from the vaginal mucosal epithelium from a vaginal smear was recorded. Results from these assessments are presented in Tables 6, 7, and 8.

TABLE 6

Primary Efficacy Analysis Results of Change from Baseline (Screening) to Day 15 in the Maturation Index of the Vaginal Smear (Percent Parabasal Cells)						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference	Estradiol 10 µg vs. Placebo P-value
Intent-to-Treat	N	24	24	—	—	—
	Least-Squares Mean	-54.4	-4.80	-49.6	(-60.4, -38.8)	<0.0001
	Mean ± SD	-53.8 ± 39.7	-5.4 ± 22.3	—	—	—
	Median	-60.0	-5.0	—	—	—
	Min, Max	-100.0, 0.0	-60.0, 60.0	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

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TABLE 7

Primary Efficacy Analysis Results of Change from Baseline (Screening) to Day 15 in the Maturation Index of the Vaginal Smear (Superficial Cells)						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference	Estradiol 10 µg vs. Placebo P-value
Intent-to-Treat	N	24	24	—	—	—
	Least-Squares Mean	35.2	8.75	26.5	(15.4, 37.6)	0.0002
	Mean ± SD	35.2 ± 26.4	8.8 ± 18.7	—	—	—
	Median	40.0	0.0	—	—	—
	Min, Max	0.0, 80.0	0.0, 90.0	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANOVA with treatment as a fixed effect.

²P-value for treatment comparison from ANOVA with treatment as a fixed effect.

TABLE 8

Primary Efficacy Analysis Results of Change from Baseline (Screening) to Day 15 in the Maturation Index of the Vaginal Smear (Intermediate Cells)						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference	Estradiol 10 µg vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-Squares Mean	18.7	-3.54	22.3	(11.1, 33.5)	0.0017
	Mean ± SD	18.5 ± 42.7	-3.3 ± 21.6	—	—	—
	Median	22.5	-5.0	—	—	—
	Min, Max	-60.0, 100.0	-60.0, 20.0	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

Change in pH Results

Vaginal pH was measured at Screening and Visit 3—End of treatment (day 15). The pH measurement was obtained by pressing a pH indicator strip against the vaginal wall. The subjects entering the study were required to have a vaginal

pH value greater than 5.0 at screening. pH values were recorded on the subject's case report form. The subjects were advised not to have sexual activity and to refrain from using vaginal douching within 24 hours prior to the measurement. Results from these assessments are presented in Table 9.

TABLE 9

Primary Efficacy Analysis Results of Change from Baseline (Screening) to Day 15 in Vaginal pH						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 µg vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-Squares Mean	-0.974	-0.339	-0.635	(-0.900, -0.368)	0.0002
	Mean ± SD	-0.917 ± 0.686	-0.396 ± 0.659	—	—	—
	Median	-1.00	-0.500	—	—	—
	Min, Max	-2.00, 0.500	-1.50, 0.500	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

Most Bothersome Symptoms Data

Subjects were asked to specify the symptom that she identified as the “most bothersome symptom.” During the screening period all of the subjects were provided with a questionnaire to self-assess the symptoms of VVA: (1) vaginal dryness; (2) vaginal or vulvar irritation, burning, or itching; (3) dysuria; (4) dyspareunia; (5) vaginal bleeding associated with sexual activity. Each symptom, with the exception of vaginal bleeding associated with sexual activity, was measured on a scale of 0 to 3, where 0=none, 1=mild, 2=moderate, and 3=severe. Vaginal bleeding asso-

ciated with sexual activity was measured in a binary scale: N=no bleeding; Y=bleeding. The subject's responses were recorded. All randomized subjects were also provided a questionnaire to self-assess the symptoms of VVA at Visit 1—Randomization/Baseline (day 1) and at Visit 3—End of the treatment (day 15). Subjects recorded their self-assessments daily in a diary and answers were collected on days 8 and 15 (end of treatment). Pre-dose evaluation results obtained at Visit 1 were considered as baseline data for the statistical analyses. Data from these assessments are presented in Tables 10 and 11.

TABLE 10

Baseline Characteristics for Vaginal Atrophy Symptoms (ITT Population)				
VVA Symptom	Statistics	Estradiol 10 µg	Placebo	Estradiol 10 µg vs. Placebo P-value ¹
Vaginal dryness	N of Subjects	24	24	—
	Mean	2.292	2.375	0.68231
Vaginal or vulvar irritation/ burning/itching	N of Subjects	24	24	—
	Mean	0.875	1.333	0.08721
Pain, burning or stinging when urinating	N of Subjects	24	24	—
	Mean	0.583	0.625	0.87681
Vaginal pain associated with sexual activity	N of Subjects ²	12	12	—
	Mean	2.083	2.333	0.54281
Vaginal bleeding associated with sexual activity	N of Subjects ²	12	12	—
	Percent ³	25.00	33.33	0.31463

¹P-value for treatment comparison from ANOVA/ANCOVA with treatment as a fixed effect and Baseline as a covariate when appropriate.

²N = number of subjects sexually active at baseline.

³Percent of subjects with bleeding, evaluated using Fisher's Exact Test.

TABLE 11

Additional Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Severity of Vaginal Atrophy Symptoms						
Symptom	Statistical Method ¹	Least-Squares Mean		Difference Between Treatment Means		Estradiol 10 µg vs. Placebo P-value
		Estradiol 10 µg	Placebo	90% CI for Difference ²		
Vaginal dryness	ANCOVA	0.980	0.729	0.251	(-0.706, 0.204)	0.3597
Vaginal or vulvar Irritation/burning/ itching	ANCOVA	0.694	0.514	0.180	(-0.549, 0.189)	0.4159
Pain/Burning/ Stinging (Urination)	ANCOVA	0.391	0.359	0.032	(-0.263, 0.200)	0.8185

TABLE 11-continued

Additional Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Severity of Vaginal Atrophy Symptoms						
Symptom	Statistical Method ¹	Least-Squares Mean		Difference Between		Estradiol 10 µg vs. Placebo P-value
		Estradiol 10 µg	Placebo	Treatment Means	90% CI for Difference ²	
Vaginal pain associated with sexual activity	ANOVA	0.800	0.500	0.300	−1.033, 0.433)	0.4872

¹ANOVA model contained a fixed effect for treatment. ANCOVA added baseline as a covariate to the model.

²Confidence interval for the difference between estradiol 10 µg and Placebo treatment least-squares means.

Changes to the most bothersome symptom from the baseline was scored according to the evaluation of VVA symptoms generally set forth above. Tables 13 and 14 show a comparison between the pharmaceutical composition 1 and placebo generally for most bothersome symptom and vaginal atrophy symptom. It is noteworthy to point out that these measurement demonstrated a trend of improvement, though not statistically significant, at day 15.

TABLE 13

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Severity of the Most Bothersome VVA						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 µg vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-Squares Mean	−1.043	−1.042	−0.002	(−0.497, 0.493)	0.9951
	Mean ± SD	−1.043 ± 0.928	−1.042 ± 1.08	—	—	—
	Median	−1.00	−1.00	—	—	—
	Min, Max	−3.00, 0.00	−3.00, 0.00	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANOVA with treatment as a fixed effect.

²P-value for treatment comparison from ANOVA with treatment as a fixed effect.

TABLE 14

Additional Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Severity of Vaginal Atrophy Symptoms						
Symptom						
Symptom	Statistical Method ¹	Least-Squares Mean		Difference Between		TX-12- 004-HR vs. Placebo P-value
		TX-12- 004-HR	Placebo	Treatment Means	90% CI for Difference ²	
Dryness	ANCOVA	−0.980	−0.729	−0.251	(−0.706, 0.204)	0.3597
Irritation	ANCOVA	−0.694	−0.514	−0.180	(−0.549, 0.189)	0.4159
Pain (Sex)	ANOVA	−0.800	−0.500	−0.300	(−1.033, 0.433)	0.4872
Pain/Burning/ Stinging (Urination)	ANOVA	−0.391	−0.359	−0.032	(−0.263, 0.200)	0.8185

¹ANOVA model contained a fixed effect for treatment. ANCOVA added baseline as a covariate to the model.

²Confidence interval for the difference between TX-12-004-HR and Placebo treatment least-squares means.

With respect to the most bothersome symptoms data presented in Tables 13 and 14, the period over which the data was measured is generally considered insufficient to make meaningful conclusions. However, the trends observed as part of this study suggest that the data will show improvement of the most bothersome symptoms when data for a longer time period is collected.

The absence or presence of any vaginal bleeding associated with sexual activity was also measured as one of the most bothersome symptoms. The data for vaginal bleeding associated with sexual activity is reported in Table 15.

TABLE 15

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Vaginal Bleeding Associated with Sexual Activity					
Baseline (Randomization) and Day 15 Summary of Vaginal Bleeding					
Treatment	N*	Bleeding/No Bleeding (Success) ^a	Bleeding/ Bleeding (Failure)	No Bleeding/ Bleeding (Failure)	No Bleeding/ No Bleeding (NC)
Estradiol 10 µg	10	2 (100%)	0	0	8
Placebo	10	1 (20%)	3	1	5
P-Value for Estradiol 10 µg vs. Placebo ¹		0.1429	—	—	—

*N = Total number of patients within each treatment group who were sexually active at both Baseline and Day 15 and provided a response at both visits.
NC = No Change - not considered in the statistical comparison.

¹P-value for treatment comparison from Fisher's Exact Test.

²Percent is based on the number of subjects classified as either a Success or a Failure (N = 2 for estradiol 10 µg; N = 5 for Placebo)

Estradiol Level/Pharmacokinetics Data

In this study, the systemic exposure to estradiol following once daily intravaginal administration of estradiol 10 µg for 14 days was investigated. Descriptive statistics of the plasma estradiol concentrations taken at each sampling time and the observed C_{max} and T_{max} values were recorded in Tables 16 and 17. No statistically significant difference in the systemic concentration of estradiol 10 µg versus the placebo group was observed, which suggests the estradiol is not carried into the blood stream where it will have a systemic effect. Rather, it remains in localized tissues; the effect of estradiol is therefore believed be local to the location of administration (i.e., the vagina). The lower limits of detection of the assays used to measure the pharmacokinetic data may have affected the measured the accuracy of the pk values presented. Additional pk studies were performed with more accurate assays in Examples 8 and 9.

For the purpose of monitoring the estradiol level during the study blood samples were collected at 0.0, 1.0, 3.0, and 6.0 hours relative to dosing on day 1; prior to dosing on day 8; and prior to dosing on day 15. Efforts were made to collect blood samples at their scheduled times. Sample collection and handling procedures for measurement of estradiol blood level was performed according to procedure approved by the sponsor and principal investigator. All baseline and post-treatment plasma estradiol concentrations were determined using a validated bioanalytical (UPLC-MS/MS) methods. These data are shown in Tables 16 and 17.

TABLE 16

Descriptive Statistics of Estradiol Concentrations (pg/ml) at Each Sampling Time						
Treatment	Sampling Time					
	0 Hour	1 Hour	3 Hours	6 Hours	Pre-dose Day 8	Pre-dose Day 15
Estradiol 10 µg						
N	24	24	24	24	24	22
Mean ± SD	20.1 ± 5.74	28.7 ± 5.89	25.7 ± 5.71	23.4 ± 7.91	21.4 ± 9.28	23.4 ± 8.72
Median	20.2	28.9	24.7	22.3	20.7	20.7
Min, Max	2.63, 38.3	18.8, 43.9	19.3, 47.5	3.31, 52.3	2.09, 52.2	17.9, 54.7
Placebo						
N	26	26	26	26	25	24
Mean ± SD	20.5 ± 4.29	21.0 ± 6.14	19.0 ± 5.92	26.9 ± 17.36	29.9 ± 22.51	28.1 ± 16.80
Median	20.8	20.8	20.9	21.7	21.6	21.1
Min, Max	4.03, 29.1	3.19, 41.2	3.15, 26.9	15.1, 90.0	15.0, 116.2	14.7, 81.3

TABLE 17

Descriptive Statistics of Estradiol C_{max} and T_{max} on Day 1.				
	Estradiol 10 μ g		Placebo	
	C_{max}	T_{max}	C_{max}	T_{max}
N	24	24	26	26
Mean \pm SD	30.7 \pm 7.47	2.12 \pm 1.73	27.5 \pm 17.26	4.00 \pm 2.68
Geometric Mean	29.9	—	24.7	—
Median	29.8	1.00	22.1	6.00
Min, Max	19.7, 52.3	1.00, 6.00	15.1, 90.0	0.00, 6.00
CV %	24.3%	81.3%	62.9%	67.1%

Assessment of Vaginal Mucosa Data

The investigators rated the vaginal mucosal appearance at day 1 (pre-dose) and day 15. Vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions were evaluated according to the following degrees of severity: none, mild, moderate, or severe using scales 0 to 3, where 0=none, 1=mild, 2=moderate, and 3=severe. Results from these investigators rated assessments are presented in Tables 18, 19, 20, and 21.

TABLE 18

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Investigator's Assessment of the Vaginal Mucosa (Assessment of Vaginal Color)						
Population	Statistics	Estradiol 10 μ g	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 μ g vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-squares Mean	−0.199	−0.009	−0.191	(−0.434, 0.052)	0.1945
	Mean \pm SD	−0.333 \pm 0.565	0.125 \pm 0.741	—	—	—
	Median	0.00	0.00	—	—	—
	Min, Max	−2.00, 0.00	−1.00, 2.00	—	—	—

¹Confidence interval for the estradiol 10 μ g-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

TABLE 19

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Investigator's Assessment of the Vaginal Mucosa (Assessment of Vaginal Epithelial Integrity)						
Population	Statistics	Estradiol 10 μ g	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 μ g vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-squares Mean	−0.342	0.176	−0.518	(−0.726, −0.311)	0.0001
	Mean \pm SD	−0.417 \pm 0.584	0.250 \pm 0.442	—	—	—
	Median	0.00	0.00	—	—	—
	Min, Max	−1.00, 1.00	0.00, 1.00	—	—	—

¹Confidence interval for the estradiol 10 μ g-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

TABLE 20

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Investigator's Assessment of the Vaginal Mucosa (Assessment of Vaginal Epithelial Surface Thickness)						
Population	Statistics	Estradiol 10 μ g	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 μ g vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-squares Mean	−0.034	−0.133	0.099	(−0.024, 0.221)	0.1820
	Mean \pm SD	−0.125 \pm 0.338	−0.042 \pm 0.550	—	—	—

TABLE 20-continued

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Investigator's Assessment of the Vaginal Mucosa (Assessment of Vaginal Epithelial Surface Thickness)						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 µg vs. Placebo P-value ²
	Median	0.00	0.00	—	—	—
	Min, Max	-1.00, 0.00	-1.00, 1.00	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

TABLE 21

Primary Efficacy Analysis Results of Change from Baseline (Randomization) to Day 15 in Investigator's Assessment of the Vaginal Mucosa (Assessment of Vaginal Secretions)						
Population	Statistics	Estradiol 10 µg	Placebo	Difference Between Treatment Means	90% CI for Difference ¹	Estradiol 10 µg vs. Placebo P-value ²
Intent-to-Treat	N	24	24	—	—	—
	Least-squares Mean	-0.643	-0.274	-0.369	(-0.661, -0.076)	0.0401
	Mean ± SD	-0.792 ± 0.779	-0.125 ± 0.741	—	—	—
	Median	-1.00	0.00	—	—	—
	Min, Max	-2.00, 1.00	-2.00, 2.00	—	—	—

¹Confidence interval for the estradiol 10 µg-Placebo from ANCOVA with treatment as a fixed effect and baseline as a covariate.

²P-value for treatment comparison from ANCOVA with treatment as a fixed effect and baseline as a covariate.

Delivery Vehicle Disintegration Data

Assessment of capsule disintegration in the vagina (presence or absence) at Day 1 (6 hours after dosing) and Day 15. Results of this assessment is presented in Table 22.

TABLE 22

Capsule Disintegration State in the Vagina on Day 1 and Day 15				
	Estradiol 10 µg		Placebo	
	Day 1	Day 15	Day 1	Day 15
No evidence of capsule present	23 (95.8%)	24 (100.0%)	26 (100.0%)	24 (92.3%)
Evidence of capsule present	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Assessment not done	1 (4.2%)	0 (0.0%)	0 (0.0%)	22 (7.7%)

Serum hormone level data was collected to measure the serum concentrations of estradiol. These data were used for screening inclusion and were determined using standard clinical chemistry methods.

Appropriateness of Measurements

The selection of the efficacy measurements used in this study was based on FDA's recommendations for studies of estrogen and estrogen/progestin drug products for the treatment of moderate to severe vasomotor symptoms associated with the menopause and moderate to severe symptoms of vulvar and vaginal atrophy associated with the menopause (Food and Drug Administration, Guidance for Industry, Estrogen and Estrogen/Progestin Drug Products to Treat

35 Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms—Recommendations for Clinical Evaluation. January 2003, hereby incorporated by reference).

Standard clinical, laboratory, and statistical procedures were utilized in the trial. All clinical laboratory procedures were generally accepted and met quality standards.

40 Statistical Methods:

Efficacy:

Analysis of variance (ANOVA) was used to evaluate the change from baseline differences between the subjects receiving estradiol 10 µg and placebo capsules for all efficacy endpoints, except for vaginal bleeding, to estimate the effect size and variability of the effect. In some cases, for example, for some vaginal atrophy symptoms, the change from baseline (post dose response) was correlated with the baseline value ($p < 0.05$), so baseline was included as a covariate to adjust for this correlation (Analysis of Covariance, ANCOVA). The 90% confidence intervals on the differences between estradiol 10 µg and placebo endpoint means were determined to evaluate the effect size. The change from baseline in vaginal bleeding associated with sexual activity was evaluated in terms of the proportion of subjects who had treatment success or failure. Any subject reporting bleeding at baseline who did not report bleeding at Day 15 was considered to have been successfully treated. Any subject reporting bleeding at day 15 was considered a treatment failure, regardless of whether they reported baseline bleeding or not. Subjects reporting no bleeding at both baseline and day 15 were classified as no-change and were excluded from the statistical evaluation. The difference in the proportion of subjects with success between the two treatment groups was statistically evaluated using Fisher's Exact Test. Results of this difference in proportion are presented in Table 10.

Measurements of Treatment Compliance

Subjects were required to complete a diary in order to record treatment compliance. Diaries were reviewed for treatment compliance at day 8 and day 15 visits. A total of 45 subjects (21 subjects in the estradiol 10 µg group and 24 subjects in the placebo group) were 100% compliant with the treatment regimen.

Due to the investigative nature of the study, no adjustments were made for multiplicity of endpoints.

Safety:

The frequency and severity of all adverse events were summarized descriptively by treatment group.

Results: All forty eight (48) subjects who completed the study were included in the primary efficacy analyses. The results of efficacy analyses are presented throughout Tables 5, 6, and 7.

Conclusions

Efficacy

The two-week treatment with pharmaceutical composition 10 µg led to a statistically significant greater mean decrease in percent of parabasal cells than did placebo treatment (54% vs. 5%, $p < 0.0001$), as illustrated in Table 6. At the same time, a significantly greater mean increase in the percent of superficial cells was observed with the pharmaceutical composition (35%) than with the placebo capsules (9%), with the difference being highly statistically significant ($p = 0.0002$), as illustrated in Table 7. The difference in pH reduction between the pharmaceutical composition (0.97 units) compared to that for the placebo (0.34 units) was only slightly greater than 0.5 units, but the difference was detected as statistically significant ($p = 0.0002$), as illustrated in Table 9.

While the decrease in severity of the most bothersome symptom was essentially the same (~1 unit) for both pharmaceutical composition and placebo, the reductions in the severity of the individual symptoms of vaginal dryness, irritation and pain during sexual activity were all marginally better for the active treatment than for the placebo treatment. None of the differences between the two treatments, all of which were ≤ 0.3 units, were detected as statistically significant. There was no difference between the two treatments in regard to reduction of pain/burning/stinging during urination (~0.4 unit reduction). The length of the study was not long enough to show a separation between the most bothersome symptoms in the pharmaceutical composition and placebo. However, the trends of most bothersome symptoms suggest that with a suitable period of time, significantly significant differences between the two treatments would be observed.

The two-week treatment with estradiol 10 µg capsules showed no statistically detectable difference in regard to reduction of severity from baseline according to the investigator's assessment of vaginal color or vaginal epithelial surface thickness. Pharmaceutical composition capsules did demonstrate a statistically significant greater reduction than did placebo in severity of atrophic effects on vaginal epithelial integrity (-0.34 vs. 0.18, $p = 0.0001$) and vaginal secretions (-0.64 vs. -0.27, $p = 0.0401$).

Descriptive statistical analyses (mean, median, geometric mean, standard deviation, CV, minimum and maximum, C_{max} and T_{max}) were conducted on the estradiol concentrations at each sampling time, the peak concentration on day 1 and the time of peak concentration. Results from this assessment are presented in Tables 16 and 17.

A pharmaceutical composition comprising estradiol 10 µg outperformed placebo treatment in regard to improvement in the Maturation Index, reduction in vaginal pH, reduction in the atrophic effects on epithelial integrity and vaginal secre-

tions. The lack of statistical significance between the two treatments in regard to reduction of severity for the most bothersome symptom, and the individual vaginal atrophy symptoms of dryness, irritation, pain associated with sexual activity, and pain/burning/stinging during urination, is not unexpected given the small number of subjects in the study and the short duration of therapy. Too few subjects in the study had vaginal bleeding associated with sexual activity to permit any meaningful evaluation of this vaginal atrophy symptom.

Of the 48 subjects enrolled in the study, 45 subjects were 100% compliant with the treatment regimen. Of the remaining three subjects, one removed herself from the study due to personal reasons and the other two subjects each missed one dose due to an adverse event.

Safety

Although the Day 1 mean plasma estradiol peak concentration for the pharmaceutical composition was somewhat higher than that for the Placebo (ratio of geometric means=1.21:Test Product (estradiol 10 µg) 21%>Placebo), no statistically significant difference was determined. However, the assay methods were questionable, resulting in questionable pk data. Additional pk studies were performed in Examples 8 and 9.

There were no serious adverse events in the study.

Overall, the pharmaceutical composition comprising estradiol 10 µg was well tolerated when administered intravaginally in once daily regimen for 14 days.

Example 8: pk Study (25 µg Formulation)

A pk study was undertaken to compare the 25 µg formulation disclosed herein (Pharmaceutical Composition 3) to the RLD. The results of the pk study for estradiol are summarized in Table 23. The p values for these data demonstrate statistical significance, as shown in Table 24.

TABLE 23

Statistical Summary of the Comparative Bioavailability Data for Unscaled Average BE studies of Estradiol, Least Square Geometric Means of Estradiol, Ratio of Means and 90% Confidence Intervals, Fasting/Fed Bioequivalence Study (Study No.: ESTR-1K-500-12); Dose 25 µg estradiol						
Parameter	Test	N	RLD	N	Ratio (%)	90% C.I.
C_{max} (pg/mL)	23.0839	36	42.7024	36	54.06	44.18-66.14
AUC_{0-24} (pg · hr/mL)	89.2093	36	292.0606	36	30.54	23.72-39.34

TABLE 24

P-values for table 23		
Effect	P-Value	
	C_{max}	AUC_{0-24}
Treatment	<.0001	<.0001
Sequence	0.4478	0.5124
Period	0.4104	0.7221

As illustrated in Table 23, baseline adjusted pk data illustrates that the formulations disclosed herein unexpectedly show a 54% decrease in C_{max} and a 31% decrease in the AUC relative to the RLD. This result is desirable because the estradiol is intended only for local absorption. These data suggest a decrease in the circulating levels of estradiol

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relative to the RLD. Moreover, it is noteworthy to point out that the C_{max} and AUC levels of estradiol relative to placebo are not statistically differentiable, which suggests that the formulations disclosed herein have a negligible systemic effect. As shown in Table 24, there was no significant difference between the test and reference products due to sequence and period effects. However, there was a significant difference due to treatment effect for both C_{max} and AUC.

Pharmacokinetics for circulating total estrone, a metabolite of estradiol, is shown in Table 25. These data show that the total circulating estrone for the formulations disclosed herein resulted in a 55% decrease in the C_{max} for circulating estrone, and a 70% decrease in the AUC for circulating estrone.

TABLE 25

Statistical Summary of the Comparative Bioavailability Data for Unscaled Average BE studies of Estrone, Least Square Geometric Means, Ratio of Means and 90% Confidence Intervals, Fasting/Fed Bioequivalence Study (Study No.: ESTR-1K-500-12); Dose 25 µg estradiol						
Parameter	Test	N	RLD	N	Ratio (%)	90% C.I.
C_{max} (pg/mL)	10.7928	36	23.5794	36	45.77	32.95 to 63.59
AUC ₀₋₂₄ (pg · hr/mL)	51.2491	36	165.4664	36	30.97	19.8-48.45

TABLE 26

P-values for table 25		
Effect	C_{max}	AUC ₀₋₂₄
Treatment	0.0002	<.0001
Sequence	0.1524	0.0464
Period	0.0719	0.0118

There was a significant difference between test and reference products due to treatment effect whereas there was no significant difference due to sequence and period effects for C_{max} . For AUC, there was a significant difference between test and reference products due to treatment, sequence, and period effects.

pk for circulating total estrone sulfate is shown in Table 27. These data show that the total circulating estrone sulfate for the pharmaceutical compositions disclosed herein resulted in a 33% decrease in the C_{max} and a 42% decrease in the AUC for circulating estrone sulfate.

TABLE 27

Statistical Summary of the Comparative Bioavailability Data for Unscaled Average BE studies of Estrone Sulfate, Least Square Geometric Means of Estrone Sulfate, Ratio of Means and 90% Confidence Intervals, Fasting/Fed Bioequivalence Study (Study No.: ESTR-1K-500-12); Dose 25 µg estradiol						
Parameter	Test	N	RLD	N	Ratio (%)	90% C.I.
C_{max} (pg/mL)	490.0449	36	730.5605	36	67.08	53.84-83.57
AUC ₀₋₂₄ (pg · hr/mL)	4232.9914	36	7323.0827	36	57.80	43.23-77.29

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TABLE 28

P-values for table 27		
Effect	P-Value	
	C_{max}	AUC ₀₋₂₄
Treatment	0.0042	0.0031
Sequence	0.5035	0.9091
Period	0.1879	0.8804

There was a significant difference between test and reference products due to treatment effect whereas there was no significant difference due sequence and period effects for both C_{max} and AUC.

Example 9: pk Study (10 µg Formulation)

A pk study was undertaken to compare the 10 µg formulation disclosed herein (Pharmaceutical Composition 2) to the RLD. The results of the pk study for estradiol are summarized in Table 29-40, and FIGS. 9-14.

A pk study was undertaken to compare pharmaceutical compositions disclosed herein having 10 µg of estradiol to the RLD. The results of the pk study for estradiol are summarized in tables 29-34, which demonstrate that the pharmaceutical compositions disclosed herein more effectively prevented systemic absorption of the estradiol. Table 35 shows that the pharmaceutical compositions disclosed herein had a 28% improvement over the RLD for systemic blood concentration C_{max} and 72% AUC improvement over the RLD.

TABLE 29

Summary of Pharmacokinetic Parameters of Test product (T) of Estradiol - Baseline adjusted (N = 34)					
Pharmaco-kinetic Parameter	Arithmetic Mean ± Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (pg/mL)	15.7176 ± 7.9179	50.3761	13.9000	6.5000	49.6000
AUC ₀₋₂₄ (pg · hr/mL)	53.0100 ± 19.5629	36.9041	49.9750	24.3000	95.1500
t_{max} (hr)	1.98 ± 1.29	65.34	2.00	1.00	8.05

TABLE 30

Summary of Pharmacokinetic Parameters of Reference product (R) of Estradiol - Baseline adjusted (N = 34)					
Pharmaco-kinetic Parameter	Arithmetic Mean ± Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (pg/mL)	24.1882 ± 11.9218	49.2877	24.1500	1.0000	55.3000
AUC ₀₋₂₄ (pg · hr/mL)	163.8586 ± 72.0913	43.9960	158.0375	2.0000	304.8500
t_{max} (hr)	10.53 ± 5.58	52.94	8.06	2.00	24.00

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TABLE 31

Geometric Mean of Test Product (T) and Reference product (R) of Estradiol - Baseline adjusted (N = 34)		
Pharmacokinetic Parameter	Geometric Mean	
	Test Product (T)	Reference Product (R)
C_{max} (pg/mL)	14.3774	20.3837
AUC ₀₋₂₄ (pg · hr/mL)	49.6231	132.9218
t_{max} (hr)	1.75	9.28

TABLE 32

Statistical Results of Test product (T) versus Reference product (R) for Estradiol - Baseline adjusted (N = 34)					
Pharmacokinetic Parameter	Geometric Least Square Mean				
	Test Product (T)	Reference Product (R)	Intra Subject CV %	T/R Ratio %	90% Confidence Interval
C_{max} (pg/mL)	14.4490	20.1980	60.68	71.54*	56.82-90.08
AUC ₀₋₂₄ (pg · hr/mL)	49.7310	131.0400	70.64	37.95*	29.21-49.31

*Comparison was detected as statistically significant by ANOVA ($\alpha = 0.05$).

The pk data for total estrone likewise demonstrated reduced systemic exposure when compared to the RLD. Table 33 shows the pharmaceutical compositions disclosed herein reduced systemic exposure by 25% for C_{max} and 49% for AUC.

TABLE 33

Summary of Pharmacokinetic Parameters of Test product (T) of Estrone - Baseline adjusted (N = 33)					
Pharmaco-kinetic Parameter	Arithmetic Mean \pm Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (pg/mL)	6.8485 \pm 6.5824	96.1149	5.4000	1.3000	36.3000
AUC ₀₋₂₄ (pg · hr/mL)	34.7051 \pm 27.9541	80.5476	30.8500	3.3500	116.7500
t_{max} (hr)	9.12 \pm 8.83	96.80	4.00	1.00	24.00

TABLE 34

Summary of Pharmacokinetic Parameters of Reference product (R) of Estrone - Baseline adjusted (N = 33)					
Pharmaco-kinetic Parameter	Arithmetic Mean \pm Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (pg/mL)	8.8333 \pm 7.1469	80.9086	6.7000	2.7000	30.3000
AUC ₀₋₂₄ (pg · hr/mL)	63.0042 \pm 46.5484	73.8814	51.2800	8.8000	214.0000
t_{max} (hr)	11.16 \pm 7.24	64.95	10.00	4.00	24.00

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TABLE 35

Geometric Mean of Test Product (T) and Reference product (R) of Estrone - Baseline adjusted (N = 33)		
Pharmacokinetic Parameter	Geometric Mean	
	Test Product (T)	Reference Product (R)
C_{max} (pg/mL)	5.1507	6.9773
AUC ₀₋₂₄ (pg · hr/mL)	24.2426	48.2377
t_{max} (hr)	5.87	9.07

TABLE 36

Statistical Results of Test product (T) versus Reference product (R) for Estrone - Baseline adjusted (N = 33)					
Pharmacokinetic Parameter	Geometric Least Square Mean				
	Test Product (T)	Reference Product (R)	Intra Subject CV %	T/R Ratio %	90% Confidence Interval
C_{max} (pg/mL)	5.1620	6.9280	47.59	74.50*	61.69-89.97
AUC ₀₋₂₄ (pg · hr/mL)	24.1960	47.9020	73.66	50.51*	38.37-66.50

*Comparison was detected as statistically significant by ANOVA ($\alpha = 0.05$).

The pk data for estrone sulfate likewise demonstrated reduced systemic exposure when compared to the RLD. Table 37 shows the pharmaceutical compositions disclosed herein reduced systemic exposure by 25% for C_{max} and 42% for AUC.

TABLE 37

Summary of Pharmacokinetic Parameters of Test product (T) of Estrone Sulfate - Baseline adjusted (N = 24)					
Pharmaco-kinetic Parameter	Arithmetic Mean \pm Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (ng/mL)	13.9042 \pm 7.0402	50.6339	11.1500	1.3000	39.0000
AUC ₀₋₂₄ (ng · hr/mL)	97.9953 \pm 80.8861	82.5408	76.2750	5.1025	338.0000
t_{max} (hr)	6.33 \pm 4.56	71.93	4.00	4.00	24.00

TABLE 38

Summary of Pharmacokinetic Parameters of Reference product (R) of Estrone Sulfate - Baseline adjusted (N = 24)					
Pharmaco-kinetic Parameter	Arithmetic Mean \pm Standard Deviation	Coefficient of Variation	Median	Minimum	Maximum
C_{max} (ng/mL)	19.2542 \pm 11.3633	59.0173	15.2000	7.0000	53.7000
AUC ₀₋₂₄ (ng · hr/mL)	177.6208 \pm 166.2408	93.5931	124.0000	20.0000	683.0500
t_{max} (hr)	10.33 \pm 5.58	54.05	10.00	2.00	24.00

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TABLE 39

Geometric Mean of Test Product (T) and Reference product (R) of Estrone Sulfate - Baseline adjusted (N = 24)		
Pharmacokinetic Parameter	Geometric Mean	
	Test Product (T)	Reference Product (R)
C_{max} (ng/mL)	12.1579	16.8587
AUC_{0-24} (ng · hr/mL)	66.5996	121.5597
t_{max} (hr)	5.49	8.83

TABLE 40

Statistical Results of Test product (T) versus Reference product (R) for Estrone Sulfate - Baseline adjusted (N = 24)					
Pharmacokinetic Parameter	Geometric Least Square Mean		Intra Subject CV %	T/R Ratio %	90% Confidence Interval
	Test Product (T)	Reference Product (R)			
C_{max} (ng/mL)	12.3350	16.5470	48.02	74.55*	59.43-93.51
AUC_{0-24} (ng · hr/mL)	68.5260	118.4170	73.87	57.87*	41.68-80.35

*Comparison was detected as statistically significant by ANOVA ($\alpha = 0.05$).

While the pharmaceutical compositions and methods have been described in terms of what are presently considered to be practical and preferred embodiments, it is to be understood that the disclosure need not be limited to the disclosed embodiments. It is intended to cover various modifications and similar arrangements included within the

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spirit and scope of the claims, the scope of which should be accorded the broadest interpretation so as to encompass all such modifications and similar embodiments. This disclosure includes any and all embodiments of the following claims.

The invention claimed is:

1. A method for treating moderate to severe dyspareunia in a human subject, the method comprising: inserting about two inches into the vagina of the subject a soft gelatin capsule containing a liquid pharmaceutical composition, wherein the composition comprises about 4 μ g to about 25 μ g of estradiol and an excipient that increases the viscosity of the composition, wherein the composition has a viscosity from about 50 cP to about 1000 cP at 25° C., and wherein the composition spreads over the vaginal tissue and the estradiol is absorbed by the vaginal tissue.

2. The method of claim 1, wherein the subject is in a reclined position while inserting the soft gelatin capsule.

3. The method of claim 1, wherein the subject is in a standing position while inserting the soft gelatin capsule.

4. The method of claim 1, wherein the viscosity of the composition is from about 50 cP to about 380 cP at 25° C.

5. The method of claim 1, wherein the excipient that increases the viscosity comprises polyethylene glycol long chain saturated fatty acid esters, ethylene glycol long chain saturated fatty acid esters, or a mixture thereof.

6. The method of claim 1, wherein the composition comprises 4 μ g estradiol or 10 μ g estradiol.

7. The method of claim 1, wherein the soft gelatin capsule is inserted once daily for two weeks and twice weekly thereafter.

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